MP MASTER
MA32945

PERKIN-ELMER

RHEODYNE
INCORPORATED

Operating Instructions For Model 7125 Syringe Loading Sample Injector

1.0 INTRODUCTION

The Model 7125 Syringe Loading Sample Injector is a rotary valve designed for high performance liquid chromatography. Supplied with the valve in a separate bag are the following items (see Fig. 5B):

a) Two socket wrenches;
b) One open-end wrench;
c) Two 22cm long tubes (0.51mm I.D.) for vents;
d) Four tube fittings (threaded bushing and ferrule) for tubing connections to valve ports;
e) One 7125 needle port cleaner;
f) Two #8-32 screws for valve mounting.

The 7125 is supplied with a #22 ga needle with a CTFE hub (part #7215) which is shipped inserted in the needle port (underneath the red protective cap). This needle should be removed from the port before use of the valve, but it should be left fully inserted in the port during periods of idleness to keep the needle seal properly sized.

The standard sample loop supplied with the 7125 is the 20 microliter size (part #7022).

Following are the standard sample loops available. Each loop is supplied with fittings for direct connection to the valve.

Catalog number	Sample Loop size microliters	Tubing bore mm	inches
7020	5	.18	.007
7021	10	.30	.012
7022	20	.51	.020
7023	50	.51	.020
7024	100	.51	.020
7025	200	.76	.030
7026	500	.76	.030
7027	1000	.76	.030
7028	2000	1.00	.040
7029	5000	1.00	.040

2.0 WARRANTY

Rheodyne products are warranted against defects in materials and workmanship for a period of one year following date of shipment. Rheodyne will make repairs or replacements free of charge upon return to the factory, transportation paid, of the defective item.

3.0 THEORY OF OPERATION

The Model 7125 is a six-port sample injection valve in which loading of the sample loop is accomplished with a syringe through a needle port built into the valve shaft.

Figure 1 shows the flow diagram of the valve. The six small circles represent the ports in the valve stator (rear of injector). The two heavy arcs represent the connecting passages in the rotor seal. The larger circle represents the needle port (in the rotor seal). The needle port is used to fill the sample loop in the LOAD position. In the INJECT position the loop is switched into the solvent stream and the needle port is vented through valve port 5. Rotation of the knob through 60° switches the valve from LOAD (CCW) to INJECT (CW).

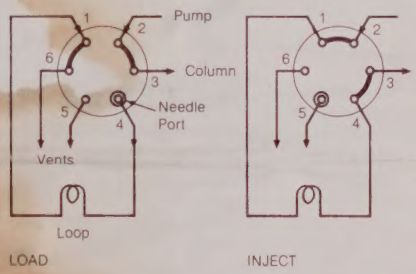

Fig. 1: Model 7125 Flow Diagram

Figure 2 shows the needle port geometry. When the syringe needle is fully inserted, the flat tip of the needle touches the flat face of the stator so that the entire volume of sample discharged from the syringe enters the stator passage which is part of the sample loop. Therefore, all of the sample discharged from the syringe becomes injected onto the column. There is no sample loss.

The flat stator face is polished alumina ceramic — a hard surface which cannot be damaged by the needle tip.

Maximum operating temperature of the Model 7125 is 80°C.

Two methods of loading the sample can be used: The complete loop filling method and the partial filling method. Following is a description of these methods.

3.0.1 FILLING THE LOOP COMPLETELY

This is the conventional method in which an excess of sample is used to insure that the sample loop is completely filled. The volume of sample is determined precisely by the loop volume and the highest degree of precision is obtained. The method is explained in Section 6.0.1.

3.0.2 PARTIALLY FILLING THE LOOP

If only small quantities of sample are available, this is the method of choice.

In this method a microsyringe is used to determine the volume of sample delivered to the loop. The loop has been previously filled with solvent from the last run and the syringe delivery causes the solvent to be displaced by the sample. The displaced solvent exits from vent tube 6.

With this method it is possible to inject samples ranging from less than one microliter up to approximately 50% of the loop capacity. Sample loops can be used on the Model 7125 ranging from 5 microliter capacity up to 5 milliliters. The partial filling method of injection is described in Section 6.0.2.

4.0 GETTING STARTED

To prepare for initial use of the Model 7125, you should follow the installation and start-up instructions in sections 5 and 6. Also be sure to read the **CAUTION** and **WARNING** notes in section 4.1. Sections 7, 8 and 9 give helpful operating suggestions, maintenance and servicing information.

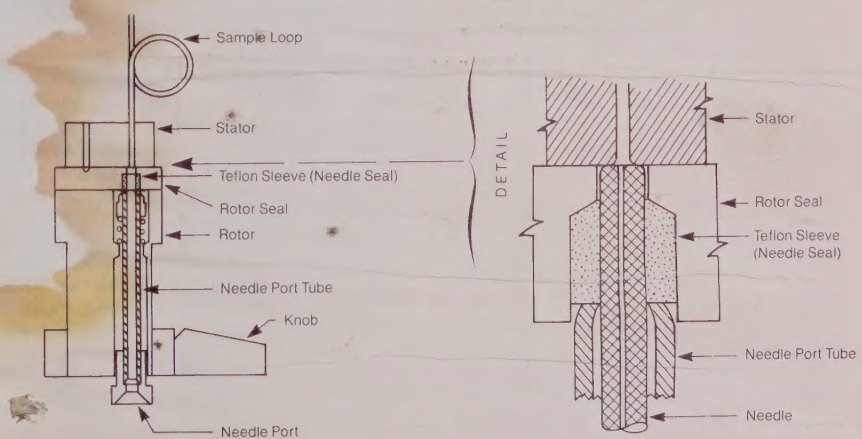

Fig. 2: Model 7125 Needle Port Geometry

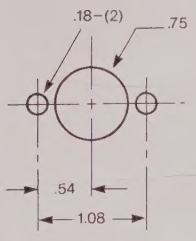

Fig. 3: Panel Holes Required (Inches)

CAUTION: To avoid liquid squirting back at you, discharge syringe slowly.

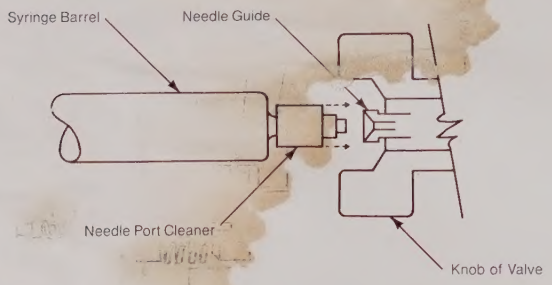

Fig 4: Use of Needle Port Cleaner

4.1 IMPORTANT SAFETY NOTICES

4.1.1 CAUTION: Be sure to use the proper size of syringe needle to avoid damage to the valve. See Section 4.2.

4.1.2 WARNING: When using the Needle Port Cleaner, discharge the syringe slowly to avoid squirting solvent back at yourself. See Section 5.1.

4.1.3 WARNING: When using sample loops larger than the 100 microliter size, protect yourself from the rapid ejection of mobile phase coming out of the needle port when the valve is turned from INJECT to LOAD. See Section 9.9.

4.2 USING PROPER SYRINGES

In both methods of sample loading it is necessary to insert a syringe needle into the needle port. In the complete filling method it is possible to use any syringe with Luer tip together with the needle supplied with the 7125. In the partial filling method, conventional low pressure microsyringes can be used. These are available from several manufacturers and should have the following needle specifications:

Needle dimensions: 0.028″ O.D. x 2″ long, without electro taper.

Point style: 90° (square end).

Failure to use needle of proper size can result in damage to the injector.

Rheodyne also supplies suitable microsyringes. Following are the part numbers:

7201 10ul Syringe
7202 25ul Syringe
7205 50ul Syringe
7210 100ul Syringe
7225 250ul Syringe
7250 500ul Syringe

5.0 INSTALLATION

a) Figure 3 shows the panel holes required for mounting the Model 7125. In addition you may need a hole to run the two vent tubes through the panel. Maximum panel thickness is 0.19″. To mount the valve, first remove the knob by loosening the two knob set screws. Use the two #8-32 screws supplied to fasten the valve to the panel.

If the Rheodyne #7161-020 Valve Position Sensing Switch is to be used, refer to the mounting instructions for that accessory.

b) When replacing the knob be sure that the two set screws are tightened securely on the two flats on the shaft. The third threaded hole in the knob is left blank. The set screws should be tightened only on flats.

c) Connect the two 22cm long tubes (supplied) to valve ports 5 and 6. Use the longer threaded bushing on port 6, and the short one on port 5. Both of these tubes should be bent so that their ends point downward to facilitate collecting the vented liquid.

To prevent siphoning effects, the outlet ends of both tubes should be at the same horizontal level as that of the needle port.

d) Connect pump to port 2 and the column tube to port 3, using the longer threaded bushing for the column tube. Leave the column disconnected from the valve during initial cleaning operations.

The use of a Rheodyne Column Inlet Filter will protect your column inlet frit from plugging from particles in the sample or from pump and valve wear particles.

5.1 USING THE NEEDLE PORT CLEANER

Connect the 7125 Needle Port Cleaner (part #7125-054) to a syringe as shown in figure 4. A syringe of at least 2 ml capacity with Luer tip (not Luer Lock) is recommended — such as Rheodyne part #7252. Use the cleaner to flush out the needle port with mobile phase with the valve in the INJECT position. To do this, push the tip of the cleaner against the conical opening of the Needle Guide and discharge the syringe slowly. Observe **WARNING 4.1.2**. The discharged liquid can squirt back at you if you are not careful.

5.2 INITIAL OPERATION

To clean out the valve and prepare it for connecting to the column, establish solvent flow through the valve. Turn valve to LOAD, then INJECT (to fill the sample loop). Use the 7125 Needle Port Cleaner to flush out the needle port with about 1 ml of mobile phase. This will leave the needle cavity filled with mobile phase. The excess solvent will flow out port 5 and leave vent tube 5 filled with solvent. Repeat the flushing step with the valve in LOAD position. This will fill vent tube 6.

Before connecting the valve to the column, make some practice injections using mobile phase. Follow the instructions in section 6. After practicing, connect the column and proceed with the chromatography.

6.0 MAKING AN INJECTION

6.0.1 FILLING THE LOOP COMPLETELY

This is the conventional method in which the volume of sample injected is precisely determined by the volume of the loop plus valve passages. Use a syringe of suitable capacity together with the #22 ga needle supplied (90° point).

a) Observe **WARNING 4.1.3** and turn valve to LOAD.

b) Fill the syringe with sample.

c) Insert the syringe needle into the needle guide all the way until the hub almost touches the needle guide. The needle tip will touch the rotor face. Do not push hard on the syringe — just be sure it is bottomed.

d) Gently discharge the syringe to completely fill the loop.

e) Leave the syringe in position and turn the valve to INJECT.

f) Remove the syringe.

g) Alternatively, use the syringe to suck up the sample from the vial into which vent tube 6 is dipped.

h) Be sure that the loading passages have been flushed with solvent after the last injection to prevent cross-contamination between runs.

i) In this method, vent tube 5 is not used. Flushing of the needle port and valve passages is done with the valve in LOAD position.

In the complete filling method, an excess of sample must be used because the fluid velocity in the sample loop tubing varies from a maximum at the tube axis to zero at the wall. As sample pushes solvent ahead of it during loading, the locus of the sample-solvent interface becomes diffuse. Solvent remains along the wall. The amount of sample in the loop approaches its maximum value asymptotically. About 2 to 3 loop volumes of sample are required to achieve 95% of maximum. This is the minimum recommended for good precision, but 5 to 10 loop volumes will provide better precision. You should determine this experimentally for yourself.

6.0.2 PARTIALLY FILLING THE LOOP

In this method, the syringe delivery determines the sample volume injected (see Section 3.0.2).

a) With valve in INJECT position, use the 7125 Needle Port Cleaner (part #7125-054) to flush out the needle port with about 1 ml of eluting solvent. This will flush out residual contamination from the previous injection. The flushing liquid will exit out of vent tube 5.

NOTE: See Section 9.3 for possible elimination of this flushing step.

b) Observe **WARNING 4.1.3** and turn valve to LOAD.

*Vespel and Teflon are trademarks of DuPont.

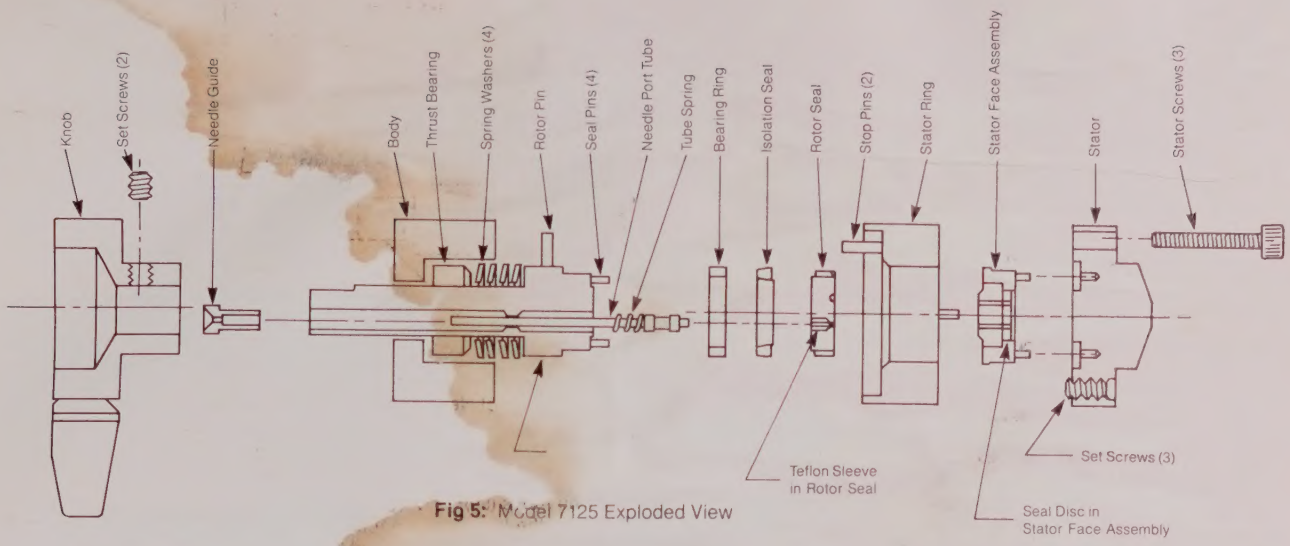

Fig. 5: Model 7125 Exploded View

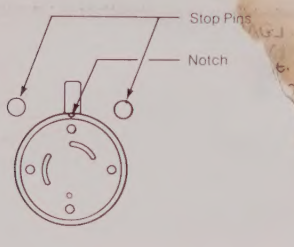

Fig. 5A
Rotor Seal Orientation (Viewed From Stator)

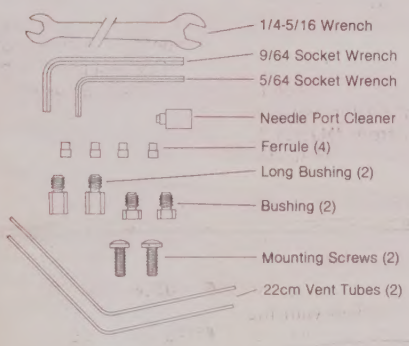

Fig. 5B: Parts supplied in separate bag

c) Load the syringe with the desired sample volume.

d) Insert syringe needle into the needle port all the way until the hub or barrel almost touches the needle guide. The needle tip will touch the stator face. Do not push hard on the syringe — just be sure it is bottomed.

e) Gently discharge the syringe contents.

f) Leave syringe in position and turn valve to INJECT.

g) Remove the syringe and leave the valve in INJECT position.

In the partial filling method, no more than half a loop volume of sample should be passed into the loop in order to maintain volumetric accuracy. With larger volumes some of the sample is lost out vent tube 6. This is because of flow velocity inequalities throughout the loop tubing.

7.0 ADJUSTING FOR HIGHER PRESSURE OPERATION

The three small set screws in the stator (see Figure 3) have been factory-set so that when the three stator screws are fully tightened, the spring force between the valve rotor and stator is sufficient to hold 34 MPa (5000 psi). If leakage is to be corrected, or if operation up to 48 MPa is to be done, proceed as follows: The three set screws should be **loosened** about 1/20 turn each (18° of rotation) and the three stator screws **tightened** an equal amount. If this new setting fails to accomplish leak-free operation, repeat the procedure by an additional 1/20 turn. Avoid excessive tightening which will accomplish nothing but increased wear of the rotor seal. If it is necessary to loosen spring tension, either to lower the operating pressure or to adjust for a new rotor seal which may be thicker than the one being replaced, reverse the above procedures; i.e., first loosen the stator screws. Then tighten the set screws.

8.0 MAINTENANCE

With normal use, the Model 7125 should give thousands of injections without trouble. The main causes of premature failure are:

a) Incorrect needle tip shape can cause the ceramic stator face to chip which then causes deep scratching of the rotor seal surface.

b) Abrasive particles in the sample which cause scratches on the rotor seal surface.

The rotor seal wears with use and is the only part that routinely needs replacement. It can be expected to last between 6 months and 2 years.

8.1 CHANGING ROTOR SEAL

To change the rotor seal proceed as follows:

a) Leave the Model 7125 attached to the panel and leave knob on. If it is necessary to remove the valve from the panel before servicing, then replace the knob after removal from panel (this simplifies the disassembly).

b) Remove the three stator screws (refer to Figure 5). Do not change the setting of the set screws in the stator at this time.

c) Remove stator, stator face, and stator ring from valve body by pulling axially to disengage the various pins.

d) Remove the rotor seal by prying it off of the four seal pins, using a screwdriver or knife blade.

e) The isolation seal and bearing ring usually are left in place because they rarely need changing.

f) Install a new rotor seal (part #7125-047) on the four seal pins by following the reassembly procedure in Section 8.2.

8.2 REASSEMBLY

Reassembly of the valve requires putting all parts back together as shown in Figure 5 while observing the following:

a) Be sure that the rotor seal is correctly oriented as shown in Figure 5A with rotor seal slots facing the stator. The needle port tube will be in line with the Teflon sleeve in the rotor seal when the rotor seal is in the correct position.

b) In replacing the stator ring, be sure that the two stop pins are replaced in their holes in the stator ring, then push the stator ring squarely onto the rotor assembly so that the stop pins enter the mating holes in the body (with the rotor pin between the two stop pins) and so that the isolation seal slips inside the stator ring without hanging up.

c) Make sure that the black seal disc is in place in the stator face assembly and that its holes are aligned with the six holes in the ceramic. This seal disc usually stays tightly in place inside the stator face assembly and should not be removed.

d) In replacing the stator face assembly on the stator, the small notch in the rim of the face assembly should be in line with port 1 of the stator. Make sure that the two pins enter the holes in stator.

e) Mount the stator and stator face assembly on the valve by pushing it onto the two pins on the stator ring and then adding the three stator screws. Be sure that the stator face assembly remains properly in position on the stator. It has a tendency to fall off; so, be careful. Tighten each screw a little at a time to keep the stator surface parallel to the stator ring surface. If the three sets screws in the stator were left unchanged, then tighten the three stator screws until all parts are held firmly in place. The three set screws will insure that the gap between stator and stator ring is uniform and exactly as it was before disassembly.

f) If the set screws need adjusting because a new rotor seal was installed or because leakage has to be stopped, refer to Section 7.0 and be sure that each set screw is turned an equal amount so that after the stator screws are retightened, the gap between the stator and stator ring is uniform all around.

g) Note that there are three threaded holes in the knob but only two set screws are used. The set screws should be tightened only on the two flats milled on the shaft.

h) After installation of a new rotor seal, it is usually necessary to form the needle seal around your syringe needle. See Section 9.2.

8.3 SUGGESTED SPARE PARTS

7125-047 Rotor Seal for 7125/7126
(expected lifetime 6 to 24 months)
Spare fittings for new tubing connections:
7010-009 Bushing
7010-011 Long Bushing
7010-062 Extra Long Bushing
7010-010 Ferrule (one required
for each make-up)

9.0 OPERATING SUGGESTIONS AND TROUBLE-SHOOTING

9.1 LEAKAGE

If liquid is observed dripping out between stator and stator ring, the stator screws should be tightened as explained in Section 7.0. Leakage out the needle port or vent tube (other than that caused by loading the loop) is caused by scratches on the rotor seal. Try tightening stator per Section 7.0, or, if this fails to stop the leakage, replace the rotor seal (part #7125-047).

NOTE: If the vent tubes from ports 5 and 6 do not have their outlet ends at the same horizontal level as the needle port, siphoning can occur which is often misinterpreted as "leakage."

9.2 NEEDLE SEAL LEAKAGE

Because the outside diameter of the syringe needle varies from syringe to syringe, the needle seal (Teflon sleeve in the rotor seal) may not immediately seal properly around a needle which is smaller than average. This will result in a loss of accuracy in loading the sample. The spring loaded needle tube will eventually reform the Teflon sleeve to make a good seal, but if you do not want to wait, do the following:

With needle removed from the needle port, push on the plastic needle guide. This will assist the spring in deforming the Teflon sleeve. Do not push so hard that the Teflon sleeve is squashed too much. A few gentle tries will produce the desired result.

To check for a proper liquid seal around the syringe needle, fill the syringe with water and slowly discharge the syringe with the injector in the LOAD position. Notice the lack of resistance to syringe discharge. Now repeat the action with the injector handle half-way between LOAD and INJECT. (The pump must be off in this position.) Now it should feel noticeably harder to discharge the syringe.

The needle seal is designed to seal around the needle only to a few psi of pressure. Since a microliter syringe can produce much higher pressure with just a small force on the plunger, do not expect the needle seal to completely prevent syringe discharge with the handle in the half-way positon.

9.3 FLUSHING BETWEEN INJECTIONS

Measurements have indicated that under proper operating conditions the residual sample left in the needle cavity and on the needle seal surface after an injection varies between 0.001 and 0.01 microliter. This represents 0.01% to 0.1% of a 10 microliter injection. If this amount of cross-contamination between successive injections is acceptable, then you do not need to flush the needle port between injections. Eliminate step (a) in Section 6.0.2.

However, it is wise to check the magnitude of cross-contamination periodically and to use the flushing step when in doubt. Conditions that can produce excessive cross-contamination are:

a) Needle is too short so that the needle tip does not touch the stator face. Minimum length from hub to needle tip should be 5.00 cm (1.97 inch).

b) Syringe is not held in place with needle bottomed in needle port while turning from LOAD to INJECT.

c) Dirt particles or needle seal shavings in the needle port are preventing the needle tip from touching the stator face.

Even when cross-contamination is not a concern, it is good practice to flush the valve (Section 5.1) about every ten injections. This prevents buildup of contamination and also keeps the needle port and vent tube 5 filled with solvent, preventing air from inadvertently entering the sample loop.

9.4 USE OF AQUEOUS BUFFERS OR SALT SOLUTIONS

To prevent the formation of salt crystals in the valve, flush out the flow passages and the needle port with water after usage of salt solutions.

9.5 USE OF HIGH pH SOLUTIONS

The standard rotor seal is Vespel, a DuPont polyimide which has exceptionally good wear resistance. However, it is susceptible to alkaline attack, deteriorating rapidly when used with solutions of pH over 10. An alternative material is available for alkaline applications (consult factory).

9.6 PLUGGED VALVE PASSAGES

If valve passages get plugged, they can be opened by removing the stator as described in Section 8.1 and cleaning the passages with a small wire (.015-inch maximum diameter).

9.7 KEEPING NEEDLE SEAL IN PROPER SHAPE

During periods of idleness of the chromatograph, such as overnight or during weekends, it is advisable to leave the 22 ga needle (supplied) fully inserted in the needle port. This will keep the needle seal (Teflon sleeve in the rotor seal) in proper shape and prevent it from being squeezed down too small.

9.8 CALIBRATING SAMPLE LOOPS

Sample loop sizes are designated by nominal values, which can vary from the actual value by as much as 20%. This is due to the ±.001" tolerance on the tubing I.D. (see table below). Since both standards and unknowns are usually analyzed with the same loop, knowledge of the absolute loop volume is rarely needed. If the actual loop volume must be known, it is best to calibrate it in place on the valve, so that the flow passages in the valve (one in the rotor and two in the stator) are also taken into account.

Tubing bore	Volume tolerance resulting from .001" bore tolerance
.012"	± 17%
.020"	± 10%
.030"	± 7%

9.9 USE OF LARGE SAMPLE LOOPS

When large sample loops are used, a few microliters of mobile phase will be expelled from the needle port and vent tube 6 when the valve is returned to LOAD. This happens because the compressed fluid in the sample loop expands when it is exposed to atmospheric pressure. Since the compressibility of most solvents is about 10^{-4} per atmosphere, the solvent in a 1-ml sample loop will expand about 20ul upon decompressing from 21 MPa (3000 psi). Observe **WARNING 4.1.3**. A small test tube or absorbent tissue can be placed at the needle port when returning the valve to LOAD, in order to safely catch the expelled liquid.

9.10 CHANGING SAMPLE LOOPS AND COLUMN CONNECTIONS

The depth of the tubing holes in the valve ports may vary slightly from port to port and from valve to valve. A fitting made up in one port may leave a dead space in another port. It is good practice, therefore, to label sample loops so that if they are removed, they will be replaced in the same orientation in the same valve. Also be sure that the column connecting tube at port 3 is made up with no dead space.

REVISION NOTICE NO. 0993-9731R

PERKIN-ELMER

INSTRUMENT DIVISION

REVISIONS TO: Instructions, Series 4 Liquid Chromatograph Microprocessor-Controlled Solvent Delivery System

EFFECTIVE DATE: April 13, 1983

PULSE DAMPER INSTALLATION INSTRUCTIONS

The Pulse Damper Sealing Screw is set in the low pressure, or "out" position for shipping purposes. While the sealing screw is in this position:

1. No pressure should be applied to the LC system ports.

2. The LC system ports cannot be isolated from atmosphere (i.e., at least one system port, such as the prime-purge valve, msut be open to prevent a vacuum or pressure condition at the pulse compensator).

3. The temperature must not exceed 60°C to 65°C maximum.

INSTALLATION

The Pulse Damper Sealing Screw must be set in the high pressure, or "in" position at installation to assure proper functioning of the pulse damper. Perform the adjustment, referring to Figure 1R, as follows:

1. Ensure that the temperature of the pulse damper is normal laboratory ambient temperature (40°C, 104°F maximum).

   Caution: Extensive damage can result if the Pulse Damper Sealing Screw is adjusted while the Series 4 Pump Module is warm from shipping or storage.

2. Check that the system ports are open to atmosphere to prevent fluid fill from exceeding the low pressure limitation.

3. Open the drain by turning the valve counterclockwise.

4. Using a 1/4-in. Allen wrench, turn the sealing screw clockwise until it reaches the bottom of its adjustment. This will require a minimumn of 120 in. lbs. (138 Kg.-cm) of torque.

When the sealing screw is in the full "in" position, the high pressure seal is achieved. In this position, the upper temperature limit is 40°C, 104°F (nominal).

STORAGE OR RESHIPMENT

If the Series 4 Pump Module is to be stored for a lengthy period of time, or if it must be reshipped, the Pulse Damper Sealing Screw should be returned to the low pressure or "out" position.

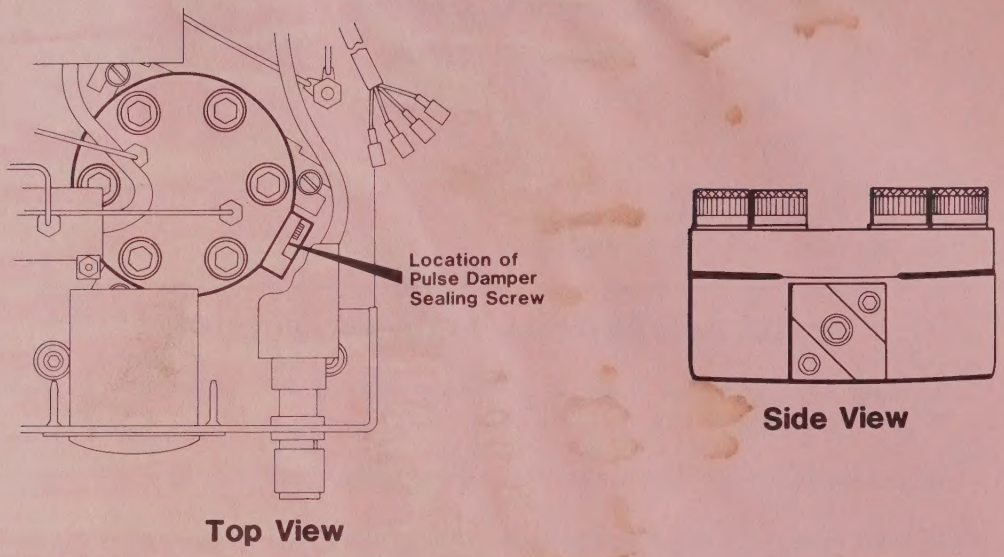

Figure 1R - Sealing Screw Location

1. Ensure that the system ports are open to atmosphere to prevent unnecessary vacuum condition in the pulse damper.

2. Using a 1/4 in. Allen wrench, loosen the Pulse Damper Sealing Screw by turning the wrench counterclockwise until the outer stop is contacted.

MICROFLOW INSTRUCTIONS

On all Series 4 instruments manufactured after October 1, 1982, a microflow switch has been added to enable the user to use the Series 4 at low flow rates with small diameter ("microbore") columns.

Setting the microflow switch to the on position reduces the actual flow to 1/10th the set and displayed value. For example, a flow rate of 0.2 ml/min. becomes an actual flow rate into the column of 0.02 ml/min. when the microflow switch is on.

The microflow pushbutton switch has a clear plastic cover to prevent accidental actuation. To actuate the switch, lift the plastic cover and press the pushbutton. A pilot light built in to the switch indicates that the switch is on. To return the Series 4 to normal operation, lift the cover and press the pushbutton. The pilot light will extinguish, indicating that the microflow switch is off.

Since the flow rate value entered in the method is divided by ten but the screen display remains unchanged, we recommend that Methods intended for use with the microflow switch indicate this in the user-entered 40 character Method name, as shown in the example on the facing page.

```
METHOD    MICROFLOW - TIMED ISOCRATIC EXAMPLE                    TIME  80.0 MINS

SECTOR    TIME    FLOW          ACN        H2O
                  MLS CURVE     A%         B%         C%         D%         CURVE

EQUIL     20.0    0.2           80.0       20.0
  1       60.0    0.2           80.0       20.0
  2        0.0
  3
  4
  5
READY TIME  80.0        PMIN  0.0   MPa   PMAX  42.0  MPa  TEMPERATURE OFF


VIAL      INJECT                  TOTAL              CURRENT                PRESSURE
          NO.                     TIME               SECTOR      TIME       MPa
                                    60.0             READY       10.0       3.0

                    FLOW          ACN        H2O

CURRENT VALUES       0.2          80.0       20.0
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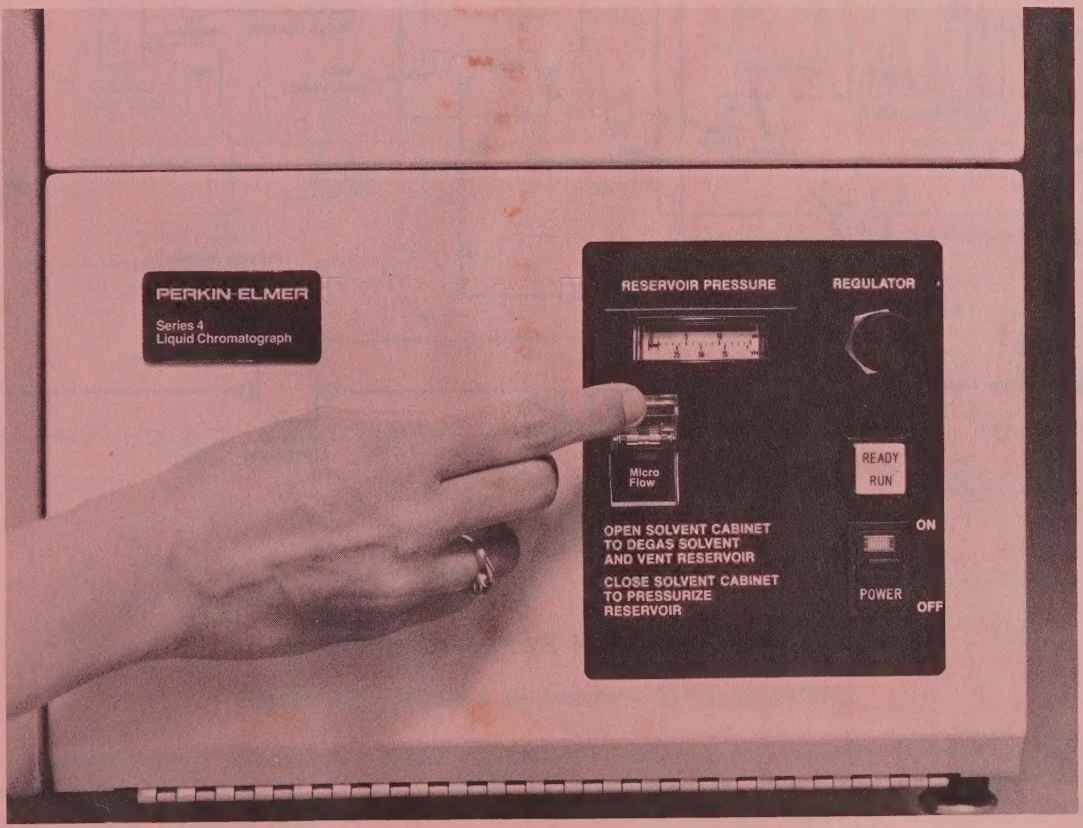


Figure 2R - Microflow Switch

MODIFICATIONS TO SERIES 4 HYDRAULICS

High Speed Gradient Liquid Chromatography requires a very brief system response time to changes in solvent composition.

Beginning with Series 4 Liquid Chromatographs shipped during February 1983, the hydraulic interconnections have been extensively modified to reduce the after pump system volume to the practical minimum. The primary purpose of the modification is to reduce the delay time at the start of the gradient when using either high speed or microbore columns.

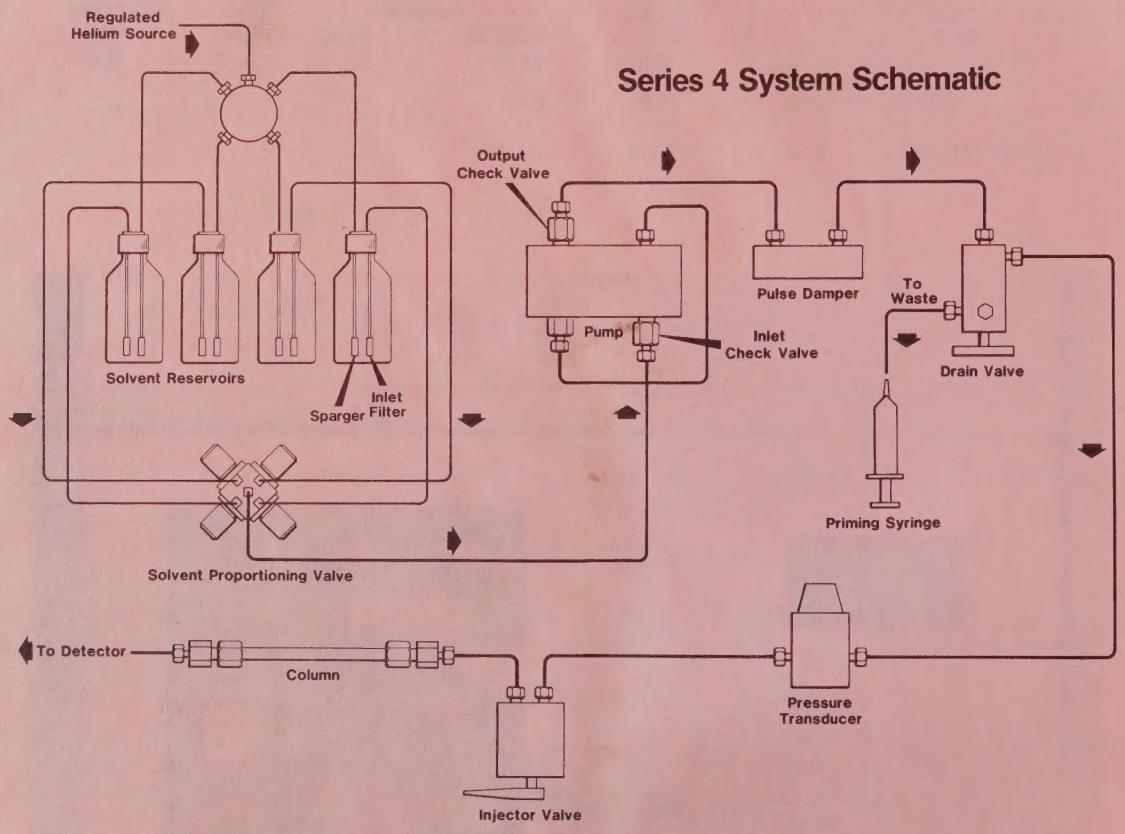


Figure 3R - Series 4 Hydraulics, as shipped

The hydraulic interconnections, as shipped from the factory are shown in Figure 3R. This is the hydraulic configuration for High Speed Gradient operation.

> **Caution:** If the Series 4 is operated in the "as shipped" configuration, solvents MUST be filtered to 0.5 microns before they are placed in the reservoir. Solvents must also be refiltered if not completely used every twenty-four hours. Water must be replaced or re-filtered every 8 hours.

Failure to adequately filter the solvents will cause plugging of the column frits. Inadequately filtered mobile phase can lead to column damage, flowcell blockage, and possible damage to LC detectors.

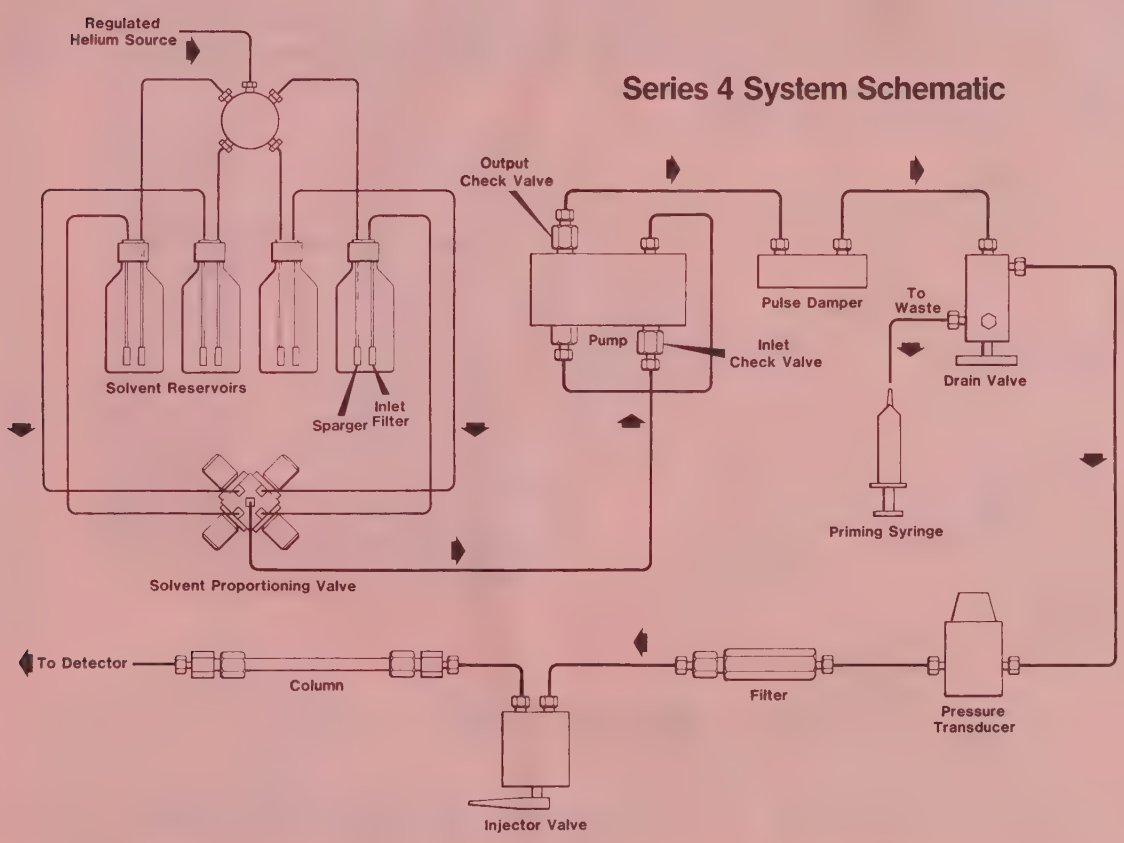


Figure 4R - Series 4 Hydraulics, Filter installed

For isocratic high speed or routine analytical separations, system volume is not a consideration because solvent composition is not changing. For these applications a 0.5 micron in-line filter is provided. The exact placement of the filter is the user's choice, but the recommended location is after the transducer and before the injector valve as shown in Figure 4R. The pump output tube, 0254-2396 may be cut and the filter spliced into it at a convenient place external to the Series 4 pump. Always observe that the flow direction arrow on the filter body is pointing in the direction of flow.

To install the filter, take the tubing end and insert it through the male nut and ferrule on the filter. Making sure that the tube has bottomed into the filter housing, tighten the male nut to between 1/4 and 1/2 turn past finger tight. Do this while holding the filter body with one wrench and turning the male nut with another wrench.

The Series 4 requires a minimum of 1.4 mega pascals (200 psi) of back pressure to allow the microprocessor to mix the solvents.

Organic mobile phase Gel Permeation LC frequently employs columns and flow rates that do not generate very high pressures. This is also true for some types of Ion Chromatography. A Back Pressure Regulator, part number 0254-0272, is provided in the Series 4 start up kit. The use of this device is mandatory whenever the total delivery pressure, as read on the Video Display Terminal, is less than 3 mega pascals (450 psi). As with the filter mentioned above, the exact location is the user's choice but the recommended location is after the transducer and before the injection valve as shown in Figure 5R. It is usually convenient to install this component external to the Series 4 pump. Use of the Back Pressure Regulator (BPR) requires the use of the filter. Always install the filter before the BPR. Always make certain that the flow arrows on both pieces point in the direction of flow.

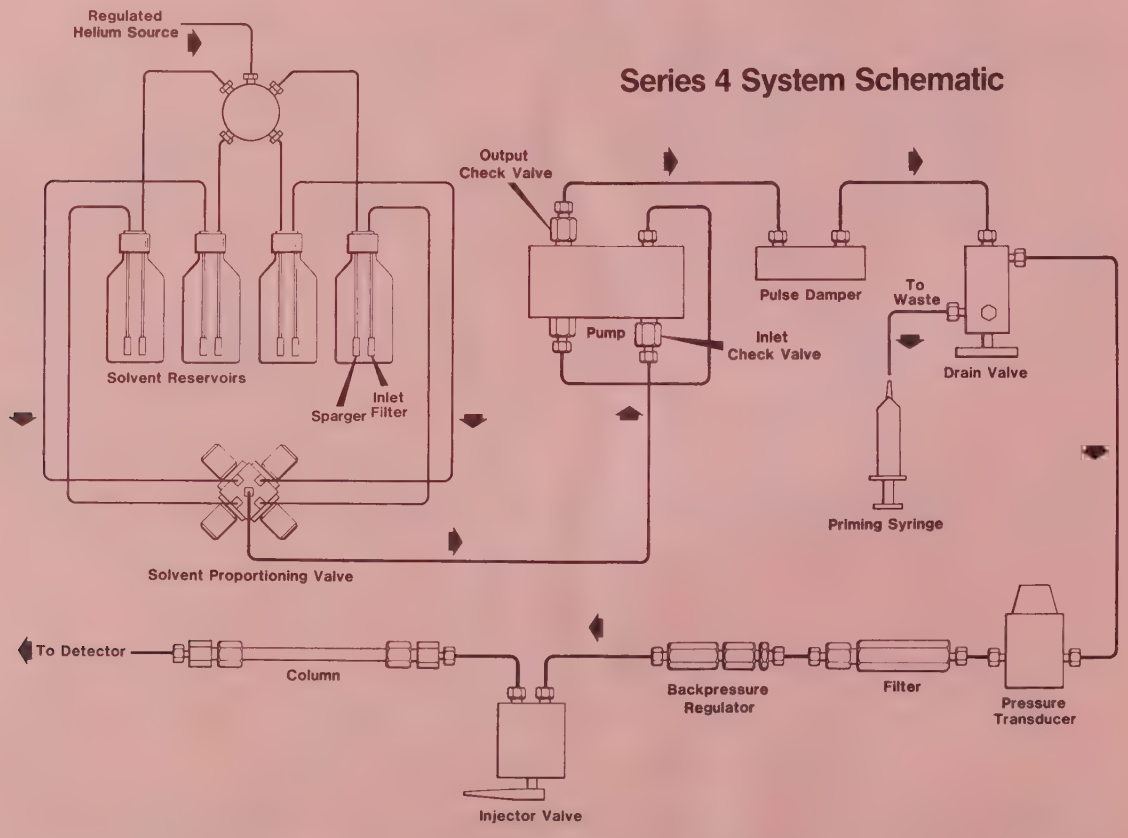


Figure 5R - Series 4 Hydraulics with all options

The following parts are now provided in the Series 4 Start Up Kit (0254-0181)

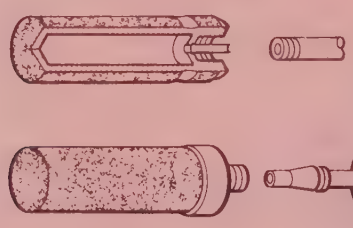
Item	P-E Part No.	Description	Qty
(1)	0087-9078	30 ml Priming Syringe	1
(2)	0254-2076	Piston Seal Backup Ring	2
(3)	0254-2077	Backup Ring Insertion Rod	1
(4)	0254-2313	Guide Bushing	1
(5)	0990-7324	High Pressure Seal	2
(6)	0990-7330	Low Pressure Seal	2
(7)	0990-7271	Allen Wrench 3/32	1
(8)	0990-7278	Allen Wrench 5/32	1
(9)	0990-7292	Allen Wrench 1/4	1
(10)	0993-9731[1]	User's Manual	1
(11)	0254-2267[1]	Injector Valve Bracket	1
(12)	0994-0022[1]	Hole Plug	2
(13)	0254-2340[1]	Blank Decal	2
(14)	0254-2396[2]	Pump Output Tube	1
(15)	0990-3980[2]	Male Compression Nut, 1/16 inch tube	3
(16)	0990-3974[2]	Ferrule, 1/16 inch Tube	3
(17)	0254-0272[2]	Back Pressure Regulator	1
(18)	0254-2286[3]	Male Gland Nut, 1/8 inch Tube	1
(19)	0990-3990[3]	Ferrule, 1/8 inch Tube	1
(20)	0250-6483[3]	PTFE Tubing 1/8 inch O.D., 1/16 I.D.	8 feet
(21)	0990-3130[3]	Back Ferrule, 1/8 inch Tube	1
(22)	0990-3129[3]	Front Ferrule, 1/8 inch Tube	1
(23)	0990-3128[3]	Female Nut, 1/8 inch Tube	1
(24)	0254-2416	Reservoir Input Line Cap	3
(25)	0990-3615	40 Micron Sparger	1
(26)	0990-7785	Sealing Washer	4
(27)	0254-1901	Solvent Line Plug	4
(28)	0990-3606	0.5 Micron Filter	1

Notes: All parts with superscript "1" are components of the injector bracket assembly. Superscript "2" denotes the componets of the Back Pressure Regulator assembly. Two items 15 and 16 are provided as user spares. All parts with superscript "3" are components of the helium inlet line for solvent degassing and reservoir pressurization.

SELECTION OF SOLVENT SPARGER

A new solvent sparger, part number 0990-3615, is being supplied in the Start Up Kit. This sparger has a pore size of 40 microns. The four spargers already installed in the Series 4 have a pore size of 10 microns. When sparging (degassing) solvents of differing viscosities and/or surface tensions the sparging rates can vary. Using this new sparger in place of the installed one will allow the user to degas the more viscous or higher surface tension solvent as effectively as the others. For example, water would be more difficult to degas than methanol, or acetonitrile, or tetrahydrofuran. Therefore, the 40 micron sparger should be used in the water reservoir.

To change spargers, firmly grip the Teflon tube and unscrew the sparger in the counterclockwise direction. Reverse the process with the new sparger. Always store the unused sparger in a clear well marked container. The two spargers are very nearly identical and confusion could easily result.



Pore	P/N
10µ	0990-3610
40µ	0990-3615
10µ	0990-7129

Figure 6R - Series 4 Filter and Spargers

ADDITIONAL FEATURES PROVIDED BY SOFTWARE ENHANCEMENTS

Beginning with Series 4 Liquid Chromatographs shipped during March 1983 the software has been enhanced to provide the following additional features.

1) Method storage capability increased from a total of 9 to a total of 15.

2) Programmable timed segments within a method increased from 5 to 9.

3) Ability to modify a method while it is running.

4) Capability to vary all solvent parameters within each timed segment.

5) Active status of system performance at all times.

6) Memory protection for up to 48 hours.

These enhancements require some slight changes in the operation of the Series 4 not covered by this manual (0993-9731). The procedures to utilize the enhancements are explained in this addendum.

INCREASED METHOD STORAGE

Storing a method in memory is still accomplished by the same procedure stated in Section 3.8 of the manual's operating summary or in Sections 4.10.5, 4.11.5 or 4.12.5 under OPERATION with the exception that the user may enter a method number from 1 to 15 instead of from 1 to 9.

NINE TIMED SEGMENTS PER METHOD

The number of timed segments in each method has been increased from a total of 5 to a total of 9. To accomodate the extra four segments the screen is automatically rewritten to a second page after the entries of the first five segments of the method have been accomplished. For operator convenience the entries for segment 5 are repeated on the display in page two of the method. After entering the parameters for segments 6 through 9 the method can be stored.

To call up the first page of the expanded method press the **RESET** and **METH** keys. In answer to the systems request for "method no." enter the number under which the method was stored or press **ENTER** without a number to bring up the first page of the current method.

To review the second page of the method press the **Y** key in response to the query, "Second Page".

MODIFICATION OF A RUNNING METHOD

It is now possible to modify a solvent program while it is actually running (the pump no longer has to be in EQUIL or SHUTDOWN). To modify a running method press the **RESET** and **MOD** keys and enter changes in any time segment beyond the one which is currently active. To modify segment one through five one can step directly to the desired sector by pressing the appropriate parameter key (e.g., T1, T5, etc.). The **ENTER** key can be

pressed to sequentially step to segments 6 through 9. After modifying the parameters in the desired sector exit from this routine by pressing the **REPEAT** and **ENTER** keys to rapidly step through the remaining sectors until an audible "beep" signals that the exit has been completed.

Frequently, it is desired to quickly change the chromatography in process (modify on the fly). In this case you will be modifying the parameters in the segment that the program is about to step to. After changing the conditions and exiting the modify routine it is not necessary to wait for the active segment to run to completion. The system can be directed to immediately go the next segment by pressing **RESET** and the $\overline{\wedge}$ (up caret) keys. To save time, storage of the modified method can be accomplished at a later time.

If the time segment which is running expires while modifications are still in process the system will hold at the conditions programmed for the end of that sector until the modify routine is exited.

ADDITIONAL VARIABLES PER SEGMENT

Former Series 4 Liquid Chromatographs allowed only two variables per timed segment. Therefore, the chromatographer had to carefully keep track of changes being made to flow rate and/or solvent composition to avoid the display of the prompt line, "TOO MANY CURVES". Section 6.5 of this manual, "How The Series 4 Counts Curves" may now be disregarded. The software enhancements included as part of this instrumentation allow (if required) the flow rate and the composition of all four solvents to be changed from the values stated in the prior segment (note, when changing the composition of solvents A,B and C; solvent D's composition will be automatically adjusted so that the four percentages total one-hundred).

ACTIVE STATUS

The Series 4 has always provided active status of important variables such as solvent composition, pressure, oven temperature etc., during a running method. This important diagnostic is now available at all times even during modification of a method so you never lose track of the system performance.

MEMORY PROTECTION

The safe memory time for methods and method chains has been extended from 6 hours to 48 hours. Stored methods can be preserved when shutting down the Series 4 overnight or in an extended power failure.

0993-9731

INSTRUCTIONS

SERIES 4

LIQUID CHROMATOGRAPH

MICROPROCESSOR-CONTROLLED SOLVENT DELIVERY SYSTEM

July 1982

PERKIN-ELMER NORWALK, CONNECTICUT, U.S.A.

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IMPORTANT

The Pulse Damper Sealing Screw is set in the low pressure, or "out" position for shipping purposes. While the sealing screw is in this position:

1. No pressure should be applied to the LC system ports.

2. The LC system ports cannot be isolated from atmosphere (i.e., all system ports plugged, or valve closed).

3. The temperature must not exceed 60° to 65°C maximum.

INSTALLATION

The Pulse Damper Sealing Screw must be set in the high pressure, or "in" position at installation to assure proper functioning of the pulse damper. Perform the adjustment as follows:

(1) Ensure that the temperature of the pulse damper is normal laboratory ambient temperature (40°C, 104°F maximum).

Caution: Extensive damage can result if the Pulse Damper Sealing Screw is adjusted while the Series 4 Pump Module is warm from shipping or storage.

(2) Check that the system ports are open to atmosphere to prevent fluid fill from exceeding the low pressure limitation.

(3) Open the drain by turning the valve counterclockwise.

(4) Using a 1/4-in. Allen wrench, turn the sealing screw clockwise until it reaches the bottom of its adjustment.

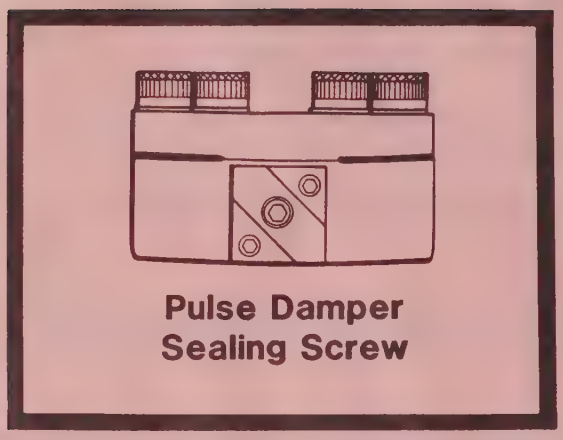
When the sealing screw is in the full "in" position, the high pressure seal is achieved. In this position, the upper temperature limit is 40°C, 104°F (nominal).

STORAGE OR RESHIPMENT

If the Series 4 Pump Module is to be stored for a lengthy period of time, or if it must be reshipped, the Pulse Damper Sealing Screw should be returned to the low pressure, or "out" position.

(1) Ensure that the system ports are open to atmosphere to prevent unnecessary vacuum condition in the pulse damper.

(2) Using a 1/4 in Allen wrench, loosen the Pulse Damper Sealing Screw by turning the wrench counterclockwise until the outer stop is contacted.



Pulse Damper
Sealing Screw

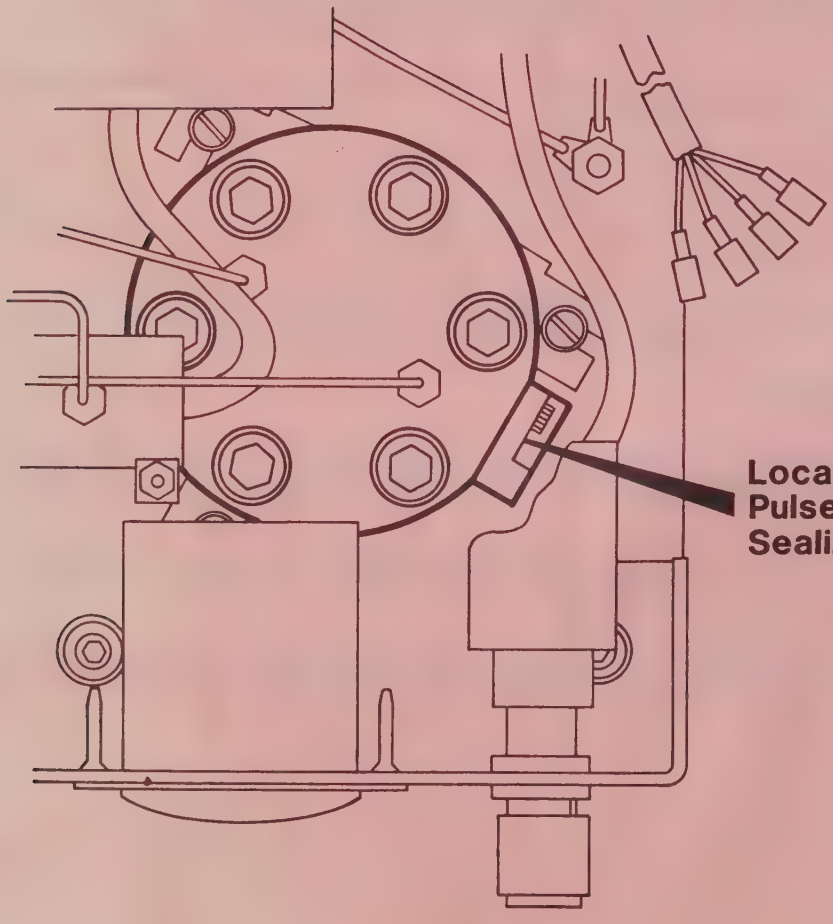


TABLE OF CONTENTS

Section		Page
1	INTRODUCTION	1-1
2	HOW TO USE THIS MANUAL	2-1
2.1	How to Use This Manual	2-1
2.2	Scope of Manual	2-2
3	OPERATING SUMMARY	3-1
3.1	Setup	3-1
3.2	Generating a Method	3-1
3.3	Degassing (Sparging) the Solvents	3-1
3.4	Pressurizing the Solvents	3-2
3.5	Priming the Pump & Purging the System	3-2
3.6	Running a Method (Starting the Pump)	3-2
3.7	Stopping a Running Method (Stopping the Pump)	3-3
3.8	Storing a Method	3-3
3.9	Recalling a Stored Method to the Screen	3-4
3.10	Modifying an Existing Method	3-4
3.11	Obtaining a Directory of Memory Contents	3-5
3.12	Creating a Chain	3-5
3.13	Erasing a Chain	3-6
3.14	Modifying a Chain	3-6
3.15	Running a Chain	3-7
3.16	Stopping a Chain Run	3-7
3.17	Using the Hold and Pause Keys	3-7
3.18	Using the Column Oven	3-8
3.19	Emergency Shutdown Procedure	3-9
4	OPERATION	4-1
4.1	Introduction	4-1
4.2	The Series 4 Method	4-1
4.3	Set Up	4-1
4.4	Degassing (Sparging) the Solvents	4-4
4.5	Pressurizing the Solvents	4-5
4.6	Priming the Pump and Purging the System	4-5
4.7	Using the Password	4-7
4.8	Using the Directory	4-7
4.9	Untimed Isocratic Analysis	4-8
4.9.1	The Default Method	4-10
4.9.2	Modifying an Existing Method	4-10
4.9.3	Storing the Untimed Isocratic Method	4-15
4.9.4	Running an Untimed Isocratic Method	4-15
4.9.5	Stopping an Untimed Isocratic Method	4-16
4.9.6	Stepping to the EQUIL Sector	4-16
4.9.7	Modifying an Untimed Isocratic Method	4-17
4.10	Timed Isocratic Analysis	4-18
4.10.1	Modifying an Existing Method	4-19
4.10.2	Storing the Modified Method	4-19
4.10.3	Generating a Timed Isocratic Method	4-20
4.10.4	Parameter Entry for a Timed Isocratic Method	4-21

TABLE OF CONTENTS (Cont'd)

Section		Page
4.10.5	Storing a Timed Isocratic Method	4-24
4.10.6	Recalling a Stored Method to the Screen	4-25
4.10.7	Running the Timed Isocratic Analysis	4-25
4.10.8	Stepping to the EQUIL Sector	4-26
4.10.9	Modifying a Timed Isocratic Analysis Method	4-26
4.10.10	Stopping a Timed Isocratic Analysis	4-26
4.10.11	Using a Timed Isocratic Method in a Chain	4-27
4.11	Gradient Analysis	4-27
4.11.1	Introduction to Gradient Analysis	4-27
4.11.2	Generating a Gradient Method	4-28
4.11.3	Parameter Entry for Gradient Analysis	4-30
4.11.4	Storing a Gradient Analysis Method	4-35
4.11.5	Recalling a Stored Method to the Screen	4-35
4.11.6	Running the Gradient Analysis Method	4-36
4.11.7	Stepping to the EQUIL Sector	4-36
4.11.8	Modifying a Gradient Analysis Method	4-37
4.11.9	Using the Hold Key	4-37
4.11.10	Using the Pause Key	4-37
4.11.11	Stoppping the Gradient Analysis	4-37
4.11.12	Using a Gradient Method in a Chain	4-37
4.12	Flow Program Analysis	4-38
4.12.1	Introduction to Flow Program Analysis	4-38
4.12.2	Modifying an Existing Method	4-39
4.12.3	Generating a Flow Program Method	4-39
4.12.4	Parameter Entry for a Flow Program	4-41
4.12.5	Storing a Flow Program Analysis	4-44
4.12.6	Recalling a Stored Method to the Screen	4-45
4.12.7	Running the Flow Program	4-45
4.12.8	Stepping to the EQUIL Sector	4-46
4.12.9	Modifying a Flow Program Method	4-46
4.12.10	Using the Hold Key	4-46
4.12.11	Using the Pause Key	4-46
4.12.12	Stopping the Flow Program Analysis	4-47
4.12.13	Using a Flow Program Method in a Chain	4-47
4.13	Combining A Gradient Program With A Flow Program	4-47
4.13.1	Parameter Entry for a Combined Gradient Program and Flow Program	4-47
4.13.2	Two Curves Per Sector	4-50
4.14	Chaining Methods Together For Unattended Analyses	4-51
4.14.1	Generating a Chain	4-51
4.14.2	Storing the Chain	4-53
4.14.3	Running a Chain	4-54
4.14.4	Stopping a Chain Run	4-55
4.14.5	Recalling a Chain Program	4-55

TABLE OF CONTENTS (Cont'd)

Section		Page
4.14.6	Modifying a Chain	4-55
4.15	Operating Without Solvent Pressurization	4-56
4.16	Operating With Duplicate Solvents For Longer Operation	4-56
4.17	Solvent Reservoir Changeover	4-57
4.17.1	Cleaning the Inlet Filter and Sparger	4-57
4.17.2	Solvent Miscibility	4-58
5	CONTROLS, INDICATORS AND KEYBOARD	5-1
5.1	Series 4 Front Panel Controls	5-1
5.1.1	POWER ON/OFF Switch	5-1
5.1.2	READY/RUN Illuminated Pushbutton	5-1
5.2	Series 4 Internal Controls	5-3
5.2.1	Drain Valve Handle	5-3
5.2.2	Vent (Flush Port)	5-3
5.3	Series 4 Pressurization Controls	5-3
5.3.1	Pressure Regulator Valve	5-3
5.3.2	Pressure Gauge	5-3
5.3.3	Solvent Chamber Door Latch Knob	5-3
5.4	Series 4 Interface Control Panel	5-3
5.4.1	MANUAL RUN/AUTO Run Switch	5-3
5.4.2	Memory Protect Switch	5-4
5.4.3	Input and Output Contacts	5-4
5.5	Power Selection & System Fuse	5-5
5.5.1	Voltage Selection & System Fuse	5-5
5.5.2	Power Cord	5-6
5.5.3	Power Supply Fuse	5-6
5.6	LC Terminal - Front Panel Controls	5-7
5.6.1	ON/OFF Switch	5-7
5.6.2	Brightness Control	5-7
5.6.3	Line Key	5-7
5.6.4	Data Line Set-Up	5-8
5.6.5	Setting the Parity and the Baud Rate on the Series 4 Pump Module	5-9
5.7	LC Terminal - Rear Panel Controls	5-9
5.7.1	RS232 Connector Port	5-9
5.7.2	Printer Port	5-9
5.7.3	Power Cord	5-9
5.7.4	Fuse	5-9
5.8	Control Module Keyboard	5-10
5.8.1	General Purpose Keys	5-10
5.8.2	Mode Command Keys (Blue Keys, Upper Row)	5-12
5.8.3	Operational Command Keys (Blue Keys, Middle Row)	5-13
5.8.4	Chain Command Keys (Blue Keys, Lower Row)	5-14
5.8.5	Function Parameter Keys (White Keys)	5-15
5.9	Software-Controlled Password	5-16
5.10	Screen Layout	5-17

TABLE OF CONTENTS (Cont'd)

Section		Page
6	OPERATOR AIDS	6-1
6.1	Operating Summary	6-1
6.2	Parameter Ranges and Default Values	6-3
6.3	Error Messages	6-4
6.4	Troubleshooting	6-5
6.5	How the Series 4 Counts Curves	6-8
6.6	Warnings and Precautions	6-10
6.6.1	Radio Frequency Energy	6-10
6.6.2	Solvents with Auto-Ignition Temperatures Below 110°C	6-10
6.6.3	Electrical Hazards	6-11
6.6.4	Mechanical Hazards	6-11
6.6.5	Corrosion	6-12
6.6.6	Pressure Build-Up	6-12
6.6.7	Air Bubbles	6-12
6.6.8	Buffers	6-13
6.6.9	Solvents	6-13
6.6.10	Solvents with Low Boiling Points	6-13
6.6.11	Magnetic Door Latch	6-13
6.6.12	Helium Supply	6-13
6.7	Blank Method Pages	6-14
7	FAMILIARIZATION EXERCISES	7-1
7.1	Materials Needed	7-1
7.2	The Series 4 Method	7-1
7.3	Untimed Isocratic Analysis	7-2
7.3.1	Generating an Untimed Isocratic Analysis	7-2
7.3.2	Storing the Untimed Isocratic Method	7-7
7.3.3	Recalling the Method from Storage	7-8
7.3.4	Degassing (Sparging) the Solvents	7-8
7.3.5	Pressurizing the Solvents	7-9
7.3.6	Priming the Pump and Purging the System	7-9
7.3.7	Running the Untimed Isocratic Method	7-11
7.3.8	Modifying the Untimed Isocratic Method	7-12
7.3.9	Stopping the Pump	7-15
7.4	Timed Isocratic Analysis	7-16
7.4.1	Modifying an Existing Method	7-16
7.4.2	Storing the Timed Isocratic Method	7-17
7.4.3	Running the Timed Isocratic Method	7-18
7.5	Gradient Analysis	7-19
7.5.1	Generating a Gradient Method	7-21
7.5.2	Storing the Gradient Method	7-26
7.5.3	Running the Gradient Analysis	7-26
7.5.4	Using the Hold and Pause Keys	7-28

TABLE OF CONTENTS (Cont'd)

Section		Page
7.5.5	Stopping the Gradient Analysis	7-29
7.6	Obtaining A Directory Of Memory Contents	7-29
7.7	Chaining Methods Together For Unattended Analyses	7-30
7.7.1	Generating a Chain	7-30
7.7.2	Storing a Chain	7-32
7.7.3	Running a Chain	7-32
7.7.4	Leaving the Chain	7-33
8	SERVICE AND MAINTENANCE	8-1
8.1	Service	8-1
8.2.	Maintenance	8-1
8.2.1	Electrical Hazards	8-1
8.2.2	Mechanical Hazards	8-1
8.2.3	Routine Checks	8-1
8.2.4	Periodic Checks	8-1
8.2.5	Solvent Input Filters	8-1
8.2.6	Solvent Spargers	8-2
8.2.7	In-Line Filter	8-3
8.2.8	Injector	8-3
8.2.9	Column	8-3
8.2.10	Replacing the Check Valves	8-3
8.2.11	Piston Seal Replacement	8-9
8.2.12	Cam Wiper	8-22
8.2.13	Cutting Stainless Steel Tubing	8-23
8.2.14	Cutting Teflon Tubing	8-23
8.2.15	Stainless Steel Fittings	8-24
9	INSTALLATION	9-1
9.1	Unpacking	9-1
9.2	Parts Supplied	9-1
9.3	Series 4 Start-Up Kit	9-2
9.4	Series 4 Accessories Available	9-2
9.5	Series 4 Optional Maintenance Kit	9-3
9.6	Series 4 Spare Parts Available	9-3
9.7	Laboratory Requirements	9-4
9.7.1	Dimensions	9-4
9.7.2	Environment	9-5
9.7.3	Configuration	9-8
9.7.4	Electrical Requirements	9-8
9.8	Connections	9-8
9.9	Power Connection	9-8
9.9.1	Voltage Selection & System Fuse	9-8
9.9.2	Power Cord	9-10
9.9.3	Power Supply Fuse	9-10
9.10	Helium Supply	9-10

TABLE OF CONTENTS (Cont'd)

Section		Page
9.11	Optional Vent Line	9-10
9.12	Connections to Injector	9-12
9.13	Connections to Column	9-12
9.14	Connections to Detector	9-12
9.15	Connections to the ISS-100 Autosampler	9-13
9.16	Connection to the 420B Autosampler	9-13
9.17	Adjusting the Pulse Damper Sealing Screw	9-20
9.17.1	Installation	9-20
9.17.2	Storage or Reshipment	9-20
9.18	Set-Up of Series 4 Pump Module	9-22
9.19	Set-Up of the Control Module	9-23
9.19.1	Data Line Switches	9-23
9.19.2	Setting the Parity and the Baud Rate on the Series 4 Pump Module	9-24
9.20	Degassing (Sparging) the Solvents	9-24
9.21	Pressurizing the Solvents	9-24
9.22	Priming the Pump and Purging the System	9-24
10	OPTIONAL COLUMN OVEN	10-1
10.1	Column Oven Installation	10-1
10.2	Unpacking	10-1
10.3	Parts Supplied	10-1
10.4	Connection to Series 4 Pump Module	10-1
10.4.1	Mechanical Connection	10-1
10.4.2	Electrical Set-Up	10-1
10.4.3	AC Connection	10-1
10.5	Installing the Column Connections to Injector and Detector	10-3
10.6	Changing Column Oven Inserts	10-5
10.7	Operation	10-5
10.7.1	Timed Method Operation	10-5
10.7.2	Untimed Isocratic Method Operation	10-5
10.7.3	Chain Operation	10-7
10.8	Solvents with Auto-Ignition Temperatures Below 110°C	10-7
10.9	Maintenance	10-8
10.10	Service	10-8
11	SPECIFICATIONS	11-1
11.1	Instrument Type	11-1
11.2	Principles of Operation	11-1
11.3	System Configuration	11-1
11.4	Numerical Specifications: Series 4 Quanternary Solvent Delivery System	11-1
11.4.1	Flow Rates	11-1
11.4.2	Flow Rate Indication	11-1
11.4.3	Flow Rate Accuracy	11-1
11.4.4	Flow Rate Reproducibility	11-1
11.4.5	Maximum Pressure	11-1
11.4.6	Pressure Sensor	11-1
11.4.7.	Pressure Limits	11-2
11.4.8	Pressure Indication	11-2

TABLE OF CONTENTS (Cont'd)

Section		Page
11.4.10	Solvent Compressibility	11-2
11.4.11	Piston Displacement	11-2
11.4.12	Compositional Accuracy	11-2
11.4.13	Compositional Precision	11-2
11.4.14	Compositional Resolutions	11-2
11.4.15	Gradient Compositional Accuracy and Reproducibility	11-2
11.4.16	Solvent Storage	11-2
11.4.17	Pump Materials	11-2
11.4.18	Programmable Methods	11-2
11.4.19	Programmable Method Chaining	11-2
11.4.20	Safe Memory	11-2
11.4.21	Video Display Unit	11-3
11.4.22	Keyboard	11-3
11.4.23	Visual Display	11-3
11.4.24	Column Oven (Optional)	11-3
11.5	External Services	11-3
11.5.1	Electrical Requirements	11-3
11.5.2	Laboratory Type	11-3
11.5.3	Operator Skill	11-3
11.6	Dimensions and Weight	11-3
11.6.1	Series 4 Pump Module	11-3
11.6.2	Series 4 Pump Module with Optional Oven	11-4
11.6.3	Control Module	11-4
11.7	Configuration	11-4
11.8	Options	11-4
11.8.1	Standard Configuration	11-4
11.9	Installation	11-4

Appendices

Appendix A	LC Solvent Polarity and Property Scale
Appendix B	Ultraviolet Absorption Bands of Common Chromophores
Appendix C	Column and Solvent Selection Guide
Appendix D	Common LC Calculations
Appendix E	The General Elution Problem and Its Solution
Appendix F	Fundamental Concepts of Liquid Chromatography
Appendix G	Use of the Solvent Gradient

LIST OF ILLUSTRATIONS

Figure		Page
2-1	Series 4 with Optional Column Oven, LC-85 Detector and the ISS-100 Autosampler	2-2
4-1	Connect the RS-232 Cable Between the Series 4 and the Control Module	4-2
4-2	Helium Line and Vent Line Connections to the Series 4	4-2
4-3	Connecting the Series 4 to the Injector, Column and Detector	4-3
4-4	Series 4 Power On/Off	4-3
4-5	Control Module Power On/Off	4-4
4-6	Pressure Regulator Knob Location	4-5
4-7	Drain Valve and Flush Port Locations	4-6
4-8	Empty Directory Screen (Screen Display Seen When Power is First Turned On)	4-9
4-9	Method Generation Screen Before Any Parameters are Entered	4-9
4-10	A Sample Untimed Isocratic Method Screen Display	4-14
4-11	A Sample Timed Isocratic Method Screen Display after Parameters Have Been Entered	4-18
4-12	Gradient Curves Available on the Series 4	4-27
4-13	A Sample Screen Display for a Linear Gradient Analysis	4-29
4-14	A Sample Screen Display for a Concave Gradient Analysis	4-29
4-15	The Linear Gradient Plotted as a Percentage of Strong Solvent Against Time	4-32
4-16	Flow Curves Available on the Series 4	4-38
4-17	A Sample Screen Display for an Isocratic Flow Program	4-40
4-18	Sample Screen Display Showing a Combined Gradient and Flow Program Method	4-40
4-19	CHAIN Generation Screen Page	4-52
4-20	Sample Chain Screen Display after Entries Have Been Made and Chain Has Been Stored	4-54
4-21a	Operating with Duplicate Solvents - 2 Reservoirs Each	4-56
4-21b	Using 3 Reservoirs for one of the Solvents	4-57
5-1	Series 4 Front Panel Controls	5-1
5-2	Series 4 Internal Controls Showing Drain Valve and Vent Locations	5-2
5-3	Series 4 Pressurization Controls	5-2
5-4	Series 4 Interface Control Panel	5-4
5-5	Voltage Selection PC Board	5-6
5-6	Control Module (Front)	5-7
5-7	Control Module Data Set-Up Switches	5-8
5-8	Control Module (Rear)	5-10
5-9	The Series 4 Control Module Keyboard	5-11
5-10	Control Module Start Up Screen Display	5-16
5-11	The Control Module Screen Display Sections	5-17

LIST OF ILLUSTRATIONS (Cont'd)

7-1	Method Generation Screen Display	7-2
7-2	Screen After Method Generation is Complete.	7-7
7-3	Pressure Regulator Knob Location	7-8
7-4	Drain Value and Flush Port Locations	7-10
7-5	Chromatogram of Run Using Method of Figure 7-2 (Test #1)	7-12
7-6	Untimed Isocratic Method After Modifications	7-14
7-7	Chromatogram of Run Using Untimed Isocratic Method after Modifications	7-15
7-8	Timed Isocratic Method	7-18
7-9	Gradient Curves Available on the Series 4	7-20
7-10	Linear Gradient Shown as % of Strong Solvent Against Time	7-21
7-11	Linear Gradient Method Screen Display	7-25
7-12	Chromatogram of Run Using Linear Gradient Method	7-27
7-13	Imporoving Test 3 by Changing the Gradient	7-27
7-14	Directory Screen Display	7-29
7-15	CHAIN Screen Page	7-30
7-18	Sample CHAIN Screen Display After Entries Have Been Made and Chain Has Been Stored	7-32
8-1	Solvent Input Filter	8-2
8-2	In-Line Filter 0990-3606	8-2
8-3a	Bulkhead Connector	8-4
8-3b	Locator Screw	8-4
8-4	Output Line From Check Valve (Pump Assembly Removed from Instrument for Clarity)	8-5
8-5	Intermediate Solvent Line Fitting	8-5
8-6	Input and Output Check Valves	8-6
8-7	Input Check Valve Washer	8-7
8-8	Tightening Check Valves With a Torque Wrench	8-8
8-9	Exploded View of Piston Assembly	8-10
8-10	Positioning the Cam	8-11
8-11	Contact Switch Wires	8-11
8-12	Retaining Pins	8-12
8-13	Allen Screw Location	8-13
8-14	Supporting the Pump Head	8-13
8-15	Vertical Spring Pin	8-14
8-16	Freeing the Vertical Spring Pin	8-15
8-17	Removing the High Pressure Piston	8-15
8-18	Removing Piston Seal Collar	8-17
8-19	Metering (low pressure) Piston Guide Bushing Inside Piston Seal Collar	8-17
8-20	High Pressure Piston Seal and Back-Up Ring	8-18
8-21	Piston Seals - Exploded View	8-19
8-22	Correct Positioning of Hooks on Vertical Spring	8-20
8-23	Replacing the Vertical Pin Spring	8-20
8-24	Proper Positioning of Pump Head in Casting Notch	8-21
8-25	Cam Wiper Location	8-22
8-26	Cutting Stainless Steel Tubing	8-23
8-27	Gripping Teflon Tubing and Inserting the Ferrule	8-23
8-28	Parker-Hannifin Fittings	8-24

LIST OF ILLUSTRATIONS(Cont'd)

9-1a	Dimensions of Series 4 Pump Module with Optional Column Oven	9-5
9-1b	Dimensions of Series 4 Control Module	9-5
9-2	The Series 4 Solvent Delivery System With the ISS-100 Autosampler	9-6
9-3	RS-232 Connections Between the Series 4 Pump Module and the Control Module	9-7
9-4	Voltage Selection PC Board	9-9
9-5	Connections for Helium Supply and Optional Vent Lines	9-11
9-6	Connection to Injector	9-12
9-7	Series 4 Hydraulic Block Diagram	9-13
9-8	ISS-100 Autosampler Connection	9-14
9-9	ISS-100 Autosampler Connection to Sigma 15 - Local Interface	9-15
9-10	ISS-100 Autosampler Connection to Sigma 15 - Remote Interface	9-16
9-11	420B Autosampler Connection	9-17
9-12	420B Autosampler Connection to Sigma 15 - Local Interface	9-18
9-13	420B Autosampler Connection to Sigma 15 - Remote Interface	9-19
9-14	Pulse Damper Sealing Screw	9-21
9-15	Data Line Set-Up	9-22
9-16	Control Module ON/OFF Switch and Intensity Control	9-23
10-1	Column Oven TB4	10-2
10-2	TB5 Barrier Strip	10-2
10-3	Connector J-10	10-3
10-4	Detector Tubing Exit	10-4
10-5	Changing Column Oven Inserts	10-4
10-6	Column Oven Door Handles	10-6

1 INTRODUCTION

1 INTRODUCTION

The Series 4 Liquid Chromatograph is a compact, modular solvent delivery system for HPLC. A precise proportioning valve delivers any one of, or any combination of, up to 4 solvents to a low dead volume high speed pump which can operate at any flow rate from 0.1 to 9.9 ml/min.

The solvent reservoirs are fully enclosed in a protective interlocked cabinet so that the solvent and laboratory environments can be totally isolated from each other to provide protection from toxic and flammable materials. The operator may pressurize the solvent to 10 psi automatically to assure pumping of high vapor pressure solvents, and to degas and then maintain the solvents gas-free. Automatic degassing assures sufficient pure degassed solvent for long-run operation.

The Control Module keyboard is used to enter solvent programs which use one, two, three, or four solvents. Up to nine user-generated methods (the set of parameters used to perform a chromatographic analysis) plus the internal method can be stored in the Series 4 memory. A moving cursor leads the operator through the input steps during method setup or modification. Once the desired conditions are entered, the microprocessor electronics automatically carry out the required proportion changes, always maintaining 100% composition by blending the solvents from the reservoirs named in the method. During operation, all key parameters and changing events are continuously displayed and automatically updated on the Control Module VDU screen. Since the complete current method is always displayed at the top of the screen and the actual status of the pump is continuously displayed and automatically updated at the bottom of the screen, during a run the operator can see at a glance what is happening now and what will happen next.

With an autosampler, methods can be chained together for automated unattended analysis of a series of different samples with the proper time and solvent program selected for each. Chaining methods is also effective for developing and/or optimizing the separation of new samples by analyzing them under a variety of solvent conditions. Up to 100 samples can be analyzed in a chain.

If the optional contact column oven and the optional autosampler are installed, sample sequencing and desired column temperature may be selected automatically for the method in use.

The contact column oven utilizes spring-loaded heated inserts to provide efficient thermal contact with the column at all times. The standard insert is preslotted to accept the various analytical columns in common use (12 cm. x 1/4" O.D., 15 cm. x 1/4" O.D., 25 cm. x 1/4" O.D., 30 cm x 1/4" O.D.). Optional inserts are available for other frequently-used columns.

2 HOW TO USE THIS MANUAL

Section		Page
2.1	How to Use This Manual	2-1
2.2	Scope of Manual	2-2

2 HOW TO USE THIS MANUAL

2.1. HOW TO USE THIS MANUAL

This manual has been organized to, hopefully, be as "user friendly" as the Series 4. Sections which are used only once or infrequently, such as Installation and General Theory of the LC Separation Process are located in the back of the book so that they need not be passed through to get to the sections used routinely such as Operation and Controls, Indicators and Keyboard.

We suggest that you familiarize yourself with the Manual and your Series 4 in the following manner.

1. If you are unfamiliar with the LC separation process or would like a brief refresher of the highlights, read the Appendices.

2. Read the Introduction for a brief description of the Series 4.

3. Then read the Controls, Indicators and Keyboard Section for an overview of the controls.

4. Although the actual installation is done by a Perkin-Elmer Service Engineer the user should read the Installation section to become familiar with the process.

5. The Familiarization Exercise is designed to take you through the basic step-by-step operation of the Series 4.

6. The Operation Section is divided up by tasks as they are normally defined in the chromatography laboratory; i.e., running the pump for an indefinite time at selected mobile phase compositions in order to start the development of a new separation method. The first task is "Untimed Isocratic Operation." Once the separation is satisfactory, the parameters should be formalized in a "Timed Isocratic Method"; the second task discussed in this Section deals with this. Methods can be stored in Series 4 memory and on the "hard copy" blank Method screen pages provided in the "Operator Aids" Section of the manual. Isocratic methods must be in the timed isocratic form to be used as part of a chain. If the separation can be improved using a gradient, read the "Gradient Operation" task description for special instructions. To perform unattended analysis of a number of samples consult the "Chain Operation" task description to learn how to link methods together in a Chain.

7. The Operator Aids Section contains a Table of Parameters giving permissible ranges and default values, a Troubleshooting Guide, a list of Error Messages and instructions on how to clear them, as well as the blank Method Screen pages mentioned above.

You are urged to take advantage of the free LC Training Course available from Perkin-Elmer. Contact your local Perkin-Elmer representative to make arrangements for this training course.

2.2. SCOPE OF MANUAL

This instruction manual contains installation, operating and service instructions for the Series 4 pump module and the optional column oven. Operating instructions for the Control Module are also included.

This equipment is designed for operation by the typical chemist or laboratory technician. This manual assumes that the user has a working knowledge of the principles of liquid chromatography.

The Series 4 may be used with a variety of instrument configurations. Figure 2-1 shows the Series 4 with the optional column oven, The LC-85 Detector and the Model ISS-100 Autosampler. Other configurations to meet your particular requirements are also available. Consult your local Perkin-Elmer Representative. Detailed information on instruments used with the Series 4 is given in separate instruction manuals.

Figure 2-1 - Series 4 with Optional Column Oven, LC-85 Detector and the ISS-100 Autosampler

3 OPERATING SUMMARY

Section		Page
3.1	Setup	3-1
3.2	Generating a Method	3-1
3.3	Degassing (Sparging) the Solvents	3-1
3.4	Pressurizing the Solvents	3-2
3.5	Priming the Pump & Purging the System	3-2
3.6	Running a Method (Starting the Pump)	3-2
3.7	Stopping a Running Method (Stopping the Pump)	3-3
3.8	Storing a Method	3-3
3.9	Recalling a Stored Method to the Screen	3-4
3.10	Modifying an Existing Method	3-4
3.11	Obtaining a Directory of Memory Contents	3-5
3.12	Creating a Chain	3-5
3.13	Erasing a Chain	3-6
3.14	Modifying a Chain	3-6
3.15	Running a Chain	3-7
3.16	Stopping a Chain Run	3-7
3.17	Using the Hold and Pause Keys	3-7
3.18	Using the Column Oven	3-8
3.19	Emergency Shutdown Procedure	3-9

3 OPERATING SUMMARY

3.1. SETUP

(1) Connect the RS-232 cable between the Control Module and the Pump Module.

(2) Connect the helium line from a 100 psi (maximum) regulated source to the Pump Module solvent chamber and, if desired, connect the vent line to a hood or other suitable exhaust.

(3) Connect the Pump Module to the injector, column(s) and detector(s). Connect recorder(s) and data processing equipment as required for the analysis.

(4) Turn the power ON at the Control Module and then at the Pump Module. The power indicator on the Pump Module will light, the Control Module will "beep" and the prompt on the last line of screen display on the Control Module will say:

POWER FAILURE ENTER PASSWORD

(5) Enter a password or default the password by pressing the ENTER key.

If entered, the password will be required to Generate or Modify a Method, to Modify a Chain, or to exit from a Chain.

See Controls, Indicators and Keyboard Section for information on using the password.

3.2. GENERATING A METHOD

(1) Press the RESET key and hold it down while pressing the GEN key.

(2) Enter the required parameters at each data entry position. (See the Operation Section of this manual for a full discussion of Method Generation.)

3.3. DEGASSING (SPARGING) THE SOLVENTS

(1) Rotate the pressure regulator knob counterclockwise 3 or 4 turns.

(2) Remove and fill the reservoirs.

(3) Replace the caps on the reservoirs and place them in the solvent chamber.

(4) With the door to the solvent chamber OPEN, rotate the pressure regulator knob clockwise to start the helium.

(5) Sparge (bubble) helium through the solvent(s) for approximately 5 minutes.

3.4. PRESSURIZING THE SOLVENTS

(1) Close the door to the solvent chamber. The reservoirs will then automatically pressurize. Bubbles will gradually decrease as the pressure rises.

(2) Adjust the pressure by rotating the knob clockwise to 7-9 psi.

Note: At pressures above 10 psi the safety release valve opens.

3.5. PRIMING THE PUMP & PURGING THE SYSTEM

(1) Check the solvent level in the reservoirs to be used.

(2) Connect the priming syringe to the flush port.

(3) Press the RESET key and hold it down while pressing the PURGE key. The program will step immediately to the PURGE program. The prompt will ask which reservoir is to be used to purge.

(4) Type in the reservoir (a, b, c, or d) to be used to purge.

Note: The reservoir to be used to purge must be identified in the Method.

(5) The prompt will ask for a purge flow rate. Press the ENTER key to get the default purge flow rate of 0 (zero). The valve will audibly click open.

(6) Open the drain valve by turning the handle counterclockwise.

(7) Collect about 20 milliliters of solvent in the syringe or continue until bubbles no longer are being expelled.

(8) Make sure that the drain valve is fully closed before removing the priming syringe.

(9) Press the RESET key and hold it down while pressing the STOP key to exit from the purge program.

3.6. RUNNING A METHOD (STARTING THE PUMP)

(1) The Method to be run must be displayed on the screen. See "Recalling a Stored Method", below.

(2) On the Control Module, press the RESET key and hold it down while pressing the START key. This will start the pump. When the equilibration time has passed, the READY button will illuminate.

(3) On the Pump Module, press the RUN button when the system is stable as indicated by an acceptable detector baseline.

Note: Since an untimed isocratic method runs continuously, the RUN button is disabled in this mode.

3.7. STOPPING A RUNNING METHOD (STOPPING THE PUMP)

(1) Press the RESET key and hold it down while pressing the STOP key on the Control Module. This will abort the analysis.

3.8. STORING A METHOD

(1) Check the screen display to see that the required parameters have been entered correctly.

(2) Press the RESET key and hold it down while pressing the STORE key.

(3) The screen prompt will request:

ENTER METHOD STORAGE NUMBER

(4a) Pressing the ENTER key will cause the system to assign the next available number to the method to be stored.

If no storage number is available, the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

will appear.

To store the new Method, overwrite an existing Method.

(4b) Entering a number which has already been used to store a previous Method will cause the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

to appear.

(4c) If the Method to be overwritten has been used in a Chain, the prompt:

METHOD EXISTS IN CHAIN, OVERWRITE (Y/N)?

will appear.

(5a) Answering N will exit from the Store program. Go back to step (2) and assign a different number or use the ENTER key to assign the next available number.

(5b) Answering Y will replace the Method stored under that number with the method presently shown on the screen display.

(5c) Since Untimed Isocratic Methods cannot be used in a Chain, a Method with an infinite ready time cannot replace a Timed Isocratic Method if the Method has been used in a Chain. Attempting this will cause the prompt

UNTIMED ISOCRATIC METHODS ARE NOT ALLOWED IN CHAIN

to appear. Assign a new storage number.

3.9. RECALLING A STORED METHOD TO THE SCREEN

The Method displayed on the screen must be in the Shutdown sector to be replaced by a Method from storage.

(1) Recall the stored Method to the screen by pressing the RESET key and holding it down while pressing the METH key on the Control Module. The prompt will ask for the Method number.

(2) Type in the number of the Method to be called to the screen from storage and press the ENTER key.

3.10. MODIFYING AN EXISTING METHOD

(1) The Method to be modified must be displayed on the Control Module. If it is not presently displayed, call the Method to the screen before continuing with the instructions below.

Note: A Method may be modified only when the Pump is in EQUIL or SHUTDOWN. To step to the EQUIL Sector, press the RESET key and hold it down while pressing the Blue EQUIL key.

(2) Press the RESET key and hold it down while pressing the MOD key on the Control Module. The prompt:

SELECT SECTOR OR PRESS ENTER KEY

will appear.

(3a) Press the appropriate parameter key (e.g., T1, T5, etc.) to step directly to the time sector where the parameter to be changed is located; or

(3b) Press the ENTER key once to indicate to the Series 4 that the ENTER key will be used to step through the parameters. The Series 4 will be in the Method description data entry position. Enter a new description or default the entry by pressing the ENTER Key. The Series 4 will step to the next data entry position.

(4) When modifications are complete, use the REPEAT and ENTER keys to step through the remaining parameters and exit from the Modify mode.

(5) Store the Modified Method.

If the Method has been used in a Chain, when the user attempts to store the modified Method, the prompt:

METHOD USED IN CHAIN, OVERWRITE (Y/N)?

will appear.

(5a) To Store the Modified Method, type Y.

If a Method used in a Chain is modified, the modifications will subsequently be used in the Chain. Check to make sure modifications will be appropriate in the Chain.

(5b) To preserve the Unmodified Method, type N.

Answering N exits from the store routine. Repeat the Store Procedure, using a different storage number for the Modified Method.

3.11. OBTAINING A DIRECTORY OF MEMORY CONTENTS

(1) Press the RESET key and hold it down while pressing the DIR key. The Method screen display will be replaced by the Directory screen.

(2) To return to the previously displayed Method press the RESET key and hold it down while pressing the METH key. The prompt:

CLEAR CURRENT METHOD (Y/N)?

will appear. Answering N will return the previously displayed Method to the screen*. Answering Y will cause Series 4 to request a Method number.

(3) Type in the Method number of the method you wish to display.

Note: If you are in the process of generating or modifying a method you must complete the generation or modification before calling the Directory.

3.12. CREATING A CHAIN

Chains are used to set up an automatic sample processing sequence. Up to 9 different stored methods may be used and up to 100 samples may be entered in the Chain, with up to 9 injections of each sample.

(1) If no Chain exists, press the RESET key and hold it down while pressing the GEN key on the Control Module. The prompt:

PRESS METHOD, CHAIN, OR CE

will appear.

(2) Press the CHAIN key. The Chain screen display will appear.

(3) Enter the required sample parameters and corresponding Methods.

Note: T Ready = INF will not allow a Method to be Chained.

*Unless the displayed Method has been previously stored, answering Y to the CLEAR CURRENT METHOD (Y/N)? prompt will cause the current Method to be erased.

(4) When all entries have been made, press the RESET key and hold it down while pressing the STORE key.

3.13. ERASING A CHAIN

(1) Press the RESET key and hold it down while pressing the GEN key. The prompt:

PRESS METHOD, CHAIN, OR CE

will appear.

(2) Press the CHAIN key. The prompt:

CHAIN EXISTS, OVERWRITE (Y/N)?

will appear.

(3) Type Y and press the ENTER key.

A new Chain may now be generated by repeating the steps given for creating a Chain.

3.14. MODIFYING A CHAIN

(1) Press the RESET key and hold it down while pressing the CHAIN key. This will display the existing Chain.

(2) Press the RESET key and hold it down while pressing the MOD key. The prompt will read:

ENTER FIRST VIAL NUMBER OF LINE TO BE MODIFIED

(3) Type in the requested number. The cursor will step to the line to be changed. Use the ENTER key to step to the proper data entry position, type in the new value and press the ENTER key as many times as needed to leave the line. When modifying a line, the left arrow key may be used, at the beginning of an entry, to step back through the line being modified.

The change will be stored automatically.

To make another modification at this time, repeat steps (2) and (3) above.

To exit from the CHAIN MODIFICATION program, follow the directions of the prompt:

PRESS RESET/METH or RESET/DIR

3.15. RUNNING A CHAIN

(1) The first Method in the Chain must be displayed on the Control Module. If it is not displayed, call the first Method to the screen before continuing.

(2) Press the RESET key and hold it down while pressing the START key to start the pump.

(3) Press the RESET key and hold it down while pressing the EXEC key on the Control Module to start the Chain.

(4) When the Equilibration time has run, the READY Light on the Pump Module will light. Press the RUN key on the Pump Module unless the RUN signal will be given by an external source.

> Note: The Pump Module must receive a RUN signal from an external source (such as an Autosampler) or the RUN button must be pressed for EACH analysis in the Chain.

3.16. STOPPING A CHAIN RUN

(1) Press the RESET key and hold it down while pressing the EXIT key on the Control Module. The prompt: CHAIN EXIT (Y/N)? will appear. Answering N will cancel the exit request. Answering Y will exit from the Chain.

(2) Press the RESET key and hold it down while pressing the STOP key on the Control Module. This will abort the analysis in progress if the Chain has been stopped.

Note: If a Chain is stopped before completion, the entire chain must be rerun or the Chain must be modified to delete the Methods which are not to be rerun from the Chain. The Chain must always begin with the first Method in the Chain. It is not possible to exit from a Chain and subsequently re-enter the Chain at the exit point.

If a Chain should be stopped temporarily, the PAUSE key, rather than the EXIT key should be used.

3.17. USING THE HOLD AND PAUSE KEYS

(1) Press the RESET key and hold it down while pressing the HOLD or PAUSE key on the Control Module. The PAUSE key will pause the pump and the internal clocks on the Series 4. The HOLD key will stop the internal clocks, but the pump will continue to run.

(2) To continue the analysis, press the RESET key and hold it down while pressing the same HOLD or PAUSE keys used to stop the system. This will restart the pump and/or the internal clocks on the Series 4.

Note: The HOLD and PAUSE keys are latching keys. The same key must be pressed to restart the system as was used to stop it.

3.18. USING THE COLUMN OVEN

When the column oven is connected, the TEMPERATURE parameter will be requested when the Series 4 Method is generated.

If this entry is defaulted in the Method, the temperature control will be OFF.

If the oven is to be used, a value between 35°C and 99°C should be entered in the Method. If the value entered is less than 35°C, but more than 9°C, the temperature control will default to a power OFF condition.

TIMED MODES

When a timed run is programmed with a heated column, after the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **Caution:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

UNTIMED ISOCRATIC MODE

If a heated oven is to be used with an untimed isocratic run, there is no automatic cool-down sequence to protect the column. The operator must modify the Method without stopping the pumps to turn off the oven and then must allow the solvent to continue to flow for a sufficient time to cool down the column.

To provide a cool-down sequence to protect the column after running in the untimed isocratic mode:

(1) Press the RESET key and hold it down while pressing the EQUIL key. This will step to the equilibration sector.

> **Caution:** DO NOT STEP TO THE SHUTDOWN SECTOR TO MODIFY THE TEMPERATURE.

(2) Press the RESET key and hold it down while pressing the MODIFY key.

(3) Press the TEMP parameter key to step directly to the temperature parameter.

(4) Type 10* and press the enter key. This will enter 10°C, which will turn the oven OFF.

(5) Allow the solvent to flow for a sufficient period of time to cool the column or remove the column to protect it before using the STOP key to stop the pump.

CHAINING METHODS WHICH USE THE OVEN

All Methods which do not use the oven temperature control should precede those which use temperatures between 35°C and 99°C. Methods which use the oven should be arranged so that the temperature increases as the Chain progresses.

*Any value between 10 and 34 may be used.

3.19. EMERGENCY SHUTDOWN PROCEDURE

(1) Turn off power to the pump and control modules.

(2) Open the door to the solvent chamber to relieve the pressure.

(3) Shut off the helium supply at the helium tank.

(4) Open the oven doors.

4 OPERATION

Section		Page
4.1	Introduction	4-1
4.2	The Series 4 Method	4-1
4.3	Set Up	4-1
4.4	Degassing (Sparging) the Solvents	4-4
4.5	Pressurizing the Solvents	4-5
4.6	Priming the Pump and Purging the System	4-5
4.7	Using the Password	4-7
4.8	Using the Directory	4-7
4.9	Untimed Isocratic Analysis	4-8
4.9.1	The Default Method	4-10
4.9.2	Modifying an Existing Method	4-10
4.9.3	Storing the Untimed Isocratic Method	4-15
4.9.4	Running an Untimed Isocratic Method	4-15
4.9.5	Stopping an Untimed Isocratic Method	4-16
4.9.6	Stepping to the EQUIL Sector	4-16
4.9.7	Modifying an Untimed Isocratic Method	4-17
4.10	Timed Isocratic Analysis	4-18
4.10.1	Modifying an Existing Method	4-19
4.10.2	Storing the Modified Method	4-19
4.10.3	Generating a Timed Isocratic Method	4-20
4.10.4	Parameter Entry for a Timed Isocratic Method	4-21
4.10.5	Storing a Timed Isocratic Method	4-24
4.10.6	Recalling a Stored Method to the Screen	4-25
4.10.7	Running the Timed Isocratic Analysis	4-25
4.10.8	Stepping to the EQUIL Sector	4-26
4.10.9	Modifying a Timed Isocratic Analysis Method	4-26
4.10.10	Stopping a Timed Isocratic Analysis	4-26
4.10.11	Using a Timed Isocratic Method in a Chain	4-27
4.11	Gradient Analysis	4-27
4.11.1	Introduction to Gradient Analysis	4-27
4.11.2	Generating a Gradient Method	4-28
4.11.3	Parameter Entry for Gradient Analysis	4-30
4.11.4	Storing a Gradient Analysis Method	4-35
4.11.5	Recalling a Stored Method to the Screen	4-35
4.11.6	Running the Gradient Analysis Method	4-36
4.11.7	Stepping to the EQUIL Sector	4-36
4.11.8	Modifying a Gradient Analysis Method	4-37
4.11.9	Using the Hold Key	4-37
4.11.10	Using the Pause Key	4-37
4.11.11	Stoppping the Gradient Analysis	4-37
4.11.12	Using a Gradient Method in a Chain	4-37
4.12	Flow Program Analysis	4-38
4.12.1	Introduction to Flow Program Analysis	4-38
4.12.2	Modifying an Existing Method	4-39

Section

4.12.3	Generating a Flow Program Method	4-39
4.12.4	Parameter Entry for a Flow Program	4-41
4.12.5	Storing a Flow Program Analysis	4-44
4.12.6	Recalling a Stored Method to the Screen	4-45
4.12.7	Running the Flow Program	4-45
4.12.8	Stepping to the EQUIL Sector	4-46
4.12.9	Modifying a Flow Program Method	4-46
4.12.10	Using the Hold Key	4-46
4.12.11	Using the Pause Key	4-46
4.12.12	Stopping the Flow Program Analysis	4-47
4.12.13	Using a Flow Program Method in a Chain	4-47
4.13	Combining A Gradient Program With A Flow Program	4-47
4.13.1	Parameter Entry for a Combined Gradient Program and Flow Program	4-47
4.13.2	Two Curves Per Sector	4-50
4.14	Chaining Methods Together For Unattended Analyses	4-51
4.14.1	Generating a Chain	4-51
4.14.2	Storing the Chain	4-53
4.14.3	Running a Chain	4-54
4.14.4	Stopping a Chain Run	4-55
4.14.5	Recalling a Chain Program	4-55
4.14.6	Modifying a Chain	4-55
4.15	Operating Without Solvent Pressurization	4-56
4.16	Operating With Duplicate Solvents For Longer Operation	4-56
4.17	Solvent Reservoir Changeover	4-57
4.17.1	Cleaning the Inlet Filter and Sparger	4-57
4.17.2	Solvent Miscibility	4-58

4. OPERATION

4.1. INTRODUCTION

This Operation Section is divided up by tasks.

The Main Divisions are: The Series 4 Method, Set Up, Degassing (Sparging) the Solvents, Pressurizing the Solvents, Priming the Pump and Purging the System, Using the Password, Using the Directory, Untimed Isocratic Analysis, Timed Isocratic Analysis, Gradient, Flow Program and combined Gradient/Flow Program Analyses and Chaining Methods for Unattended Operation. Additionally, instructions are given in this section for operating the system without pressurizing the solvents and for operating the system with 2 solvents, but with all four reservoirs filled to give additional operating time.

4.2. THE SERIES 4 METHOD

The set of parameters which are used to perform a chromatographic analysis are referred to in this manual as a Method. The Series 4 Method will include solvent composition and times and, if the optional oven is installed, temperature. The Series 4 Method, the sample preparation scheme, the chromatographic conditions, detector settings, and the data processing parameters are all part of the total Analytical Method.

We will concern ourselves here with the Series 4 (pump) Method only.

4.3. SET UP

(1) Connect the RS-232 cable between the Control Module and the Pump Module (see Figure 4-1).

(2) Connect the helium line from a 100 psi regulated source to the Pump Module input fitting (see Figure 4-2) and, if desired, connect the vent line to a hood or other suitable exhaust.

(3) Connect the Pump Module to the injector, column(s) and detector(s) as required for the analysis (see Figure 4-3).

(4) Turn the power ON at the Pump and Control Modules. (See Figures 4-4 and 4-5 for location of the ON/OFF switches.)

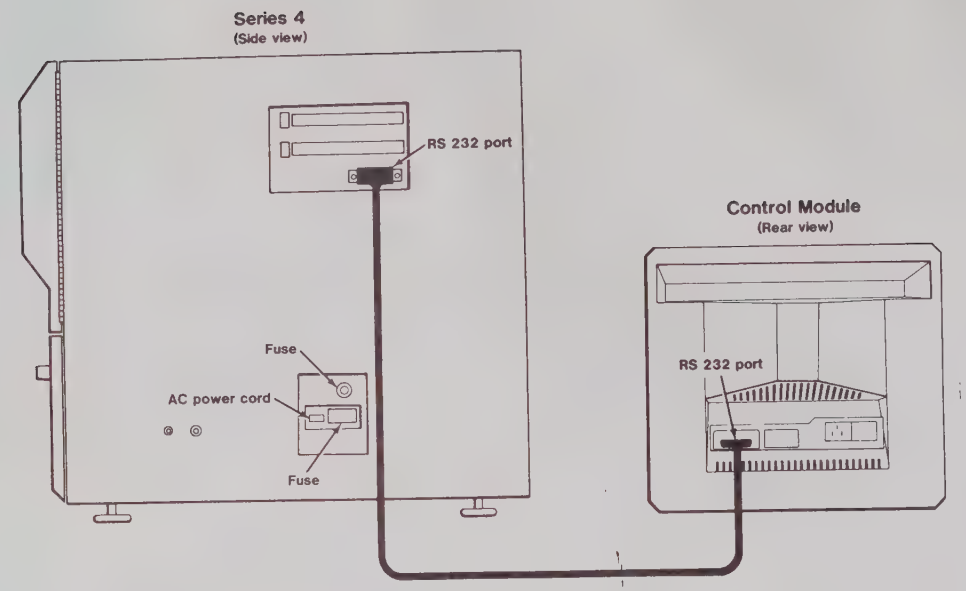


Figure 4-1 - Connect the RS-232 Cable Between the Series 4 and the Control Module

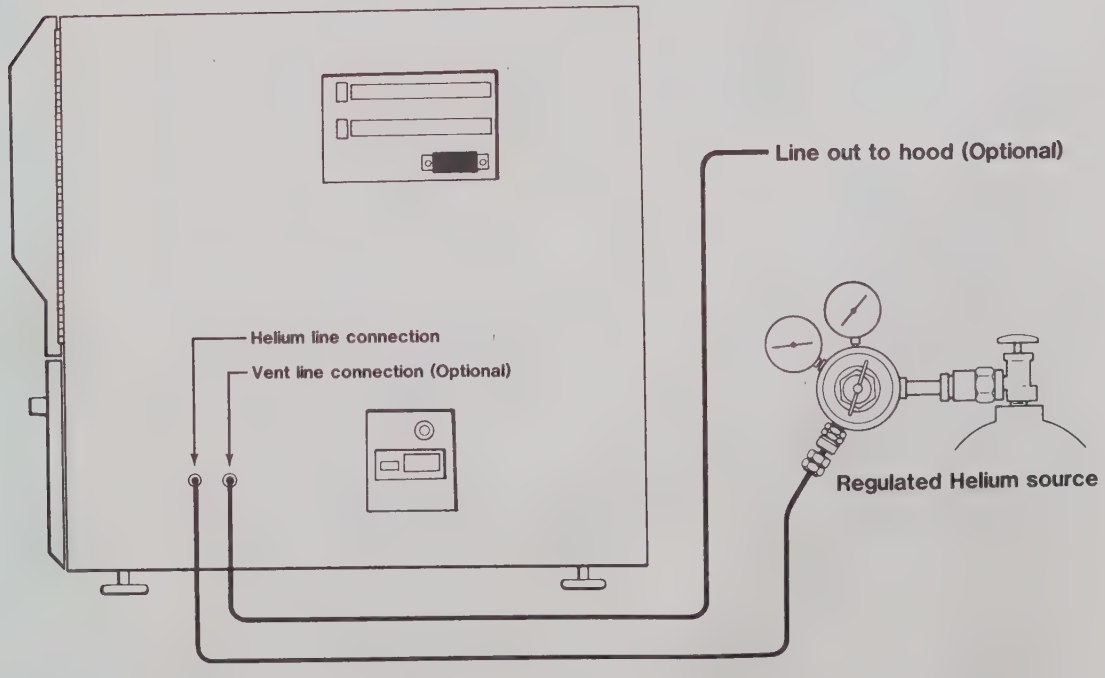


Figure 4-2 - Helium Line & Vent Line Connections to the Series 4

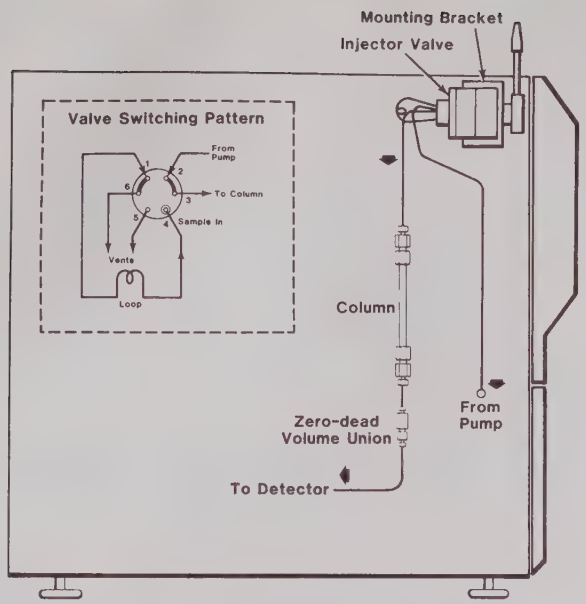


Figure 4-3 - Connecting the Series 4 To the Injector, Column and Detector

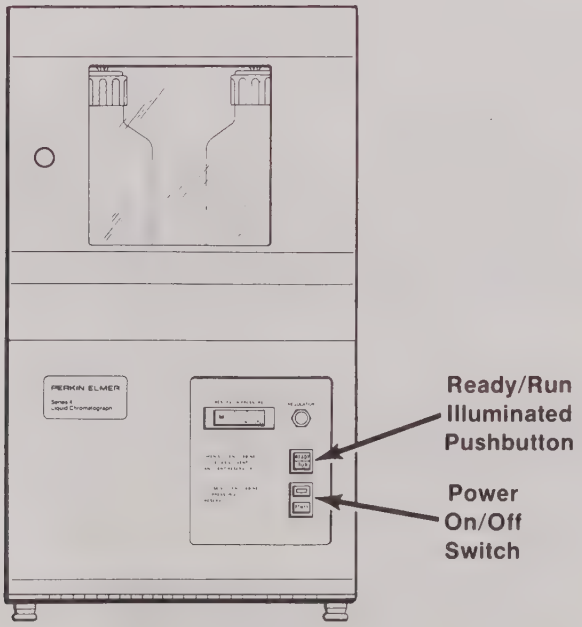


Figure 4-4 - Series 4 Power On/Off

Figure 4-5 - Control Module Power On/Off

4.4. DEGASSING (SPARGING) THE SOLVENTS

(1) Rotate the pressure regulator knob (see Figure 4-6) counterclockwise 3 or 4 times to close the valve.

(2) Remove the reservoirs from the solvent chamber. Notice that the solvent lines are labeled A, B, C, and D. Fill the Reservoirs to be used. Do not fill above 1000 µl mark. Reservoirs which will not be used, may be removed and the lines capped. The helium input line cap is P/N 0254-2416. The vent line cap is P/N 0254-2415.

(3) Replace the caps on the reservoirs, making sure that the correct solvent line leads from each reservoir (the lines are tagged A, B, C, and D). Hold the caps while turning the reservoirs to avoid twisting the lines. Place the reservoirs in the solvent chamber.

(4) With the door to the solvent chamber OPEN, rotate the pressure regulator knob clockwise to start the helium.

(5) Sparge (bubble) the solvent(s) for approximately 4 minutes.

Note: Sparging is most effective when all reservoirs are filled to approximately the same level.

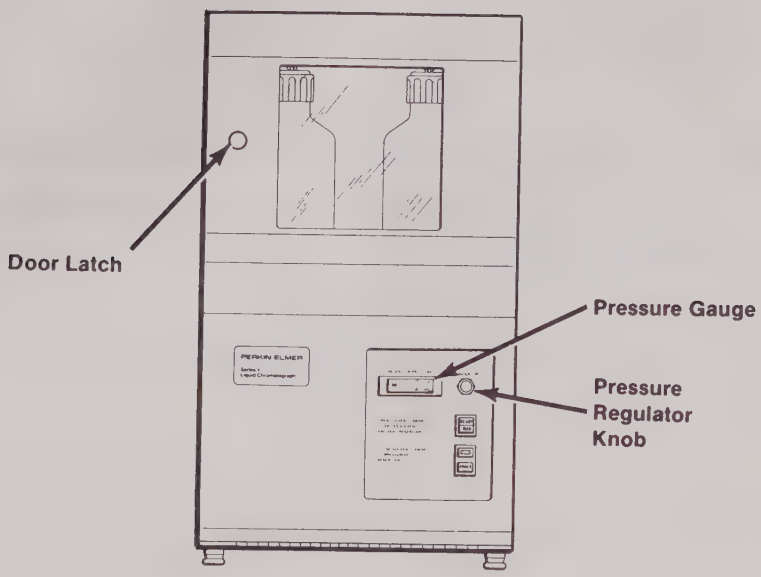


Figure 4-6 - Pressure Regulator Knob Location

4.5. PRESSURIZING THE SOLVENTS

(1) Close the door to the solvent chamber and turn the knob to latch it. The reservoirs will then automatically pressurize. The bubbles will slow and eventually stop forming.

> **Warning:** Whenever the door to the solvent chamber is opened, the solvent reservoirs depressurize automatically. This is a safety feature. Do not attempt to defeat the door interlock.

(2) Adjust the pressure by rotating the pressure regulator knob (see Figure 4-6) clockwise until the pressure gauge reads 7 to 9 psi.

Note: At pressures above 10 psi the release valve opens.

> **Important:** Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

4.6. PRIMING THE PUMP AND PURGING THE SYSTEM

Note: A Method must have been generated and must be displayed on the Screen in order for the pump to run.

(1) Open the cover to the pump compartment. (The cover to the pump compartment is hinged on the bottom.)

(2) Connect the priming syringe to the flush port (see Figure 4-7).

(3) On the Control Module press the blue RESET key and hold it down while you press the PURGE key. The prompt line will say:

RESERVOIR?

(4) Type in the letter corresponding to the reservoir to be used for the purge.

Note: If you type in anything other than the letters a, b, c, or d, the Control Module will beep and the prompt:

ERROR, CE

will appear. Type in the correct letter and press the ENTER key.

The prompt line on the VDU will say:

PURGE FLOW?

(5) To get a flow rate of 0 (zero), press the ENTER key. The valve will audibly click open.

Note: A purge flow rate is used when the column is also to be purged. Pick a purge flow appropriate to the backpressure of the column.

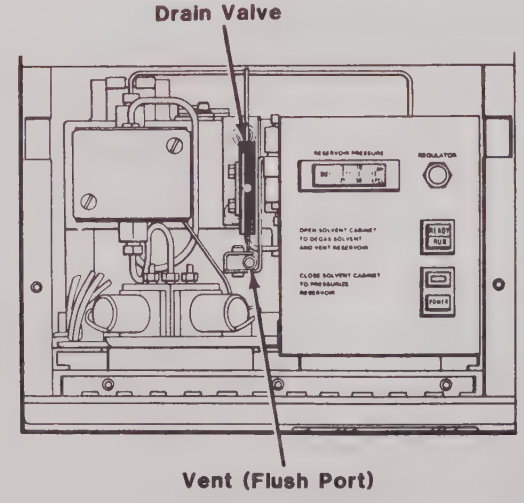


Figure 4-7 - Drain Valve and Flush Port Locations

(6) Go back to the Pump Module. Open the drain valve by turning the handle counterclockwise (see Figure 4-7 for the location of the drain valve).

(7) Collect about 20 milliliters of solvent in the syringe or continue until air bubbles have stopped coming through.

(8) Stop the flow into the syringe by turning the drain valve handle clockwise. Make sure that the valve is fully closed before removing the priming syringe.

(9) Repeat steps 1 - 8 for each reservoir to be used.

(10) Press the blue RESET key and hold it down while you press the STOP key. This will Exit from the PURGE mode.

(11) Remove the priming syringe and dispose of the solvent in an appropriate waste container.

4.7. USING THE PASSWORD

When the power is turned on, the power indicator on the Pump Module will light and the prompt line of the screen display on the Control Module will say:

POWER FAILURE ENTER PASSWORD

A two-character password may be entered at this time to prevent accidental entries from affecting the analysis. If a password is entered, then it will be required to generate or modify a Method, to modify a Chain or to exit from a Chain. The same password will be required until the power is turned off and back on, at which time the same password, a new password or no password may be entered.

When a password is entered, the Series 4 will print the first letter of the password on the prompt line but it will disappear once a second key is pressed. The second letter of the password will not be displayed. This helps to guard against accidental password entry. If the password is forgotten, it will be necessary to turn the Memory Protect Switch on and the power to the Series 4 and Control Module off, then turn the power back on and begin again.

If a password is not required, the password entry may be defaulted by pressing the ENTER key.

4.8. USING THE DIRECTORY

The directory screen display appears when the Seires 4 is first turned on.

When a Method or Chain is displayed, a directory of the contents of the Series 4 memory may be obtained at any time.

> **Note:** If you are in the process of generating or modifying a Method you must finish stepping through the Generate or Modify modes before calling the Directory.

(1) Press the RESET key and hold it down while pressing the DIR key.

(2) The Directory Screen display will replace the Method currently shown on the screen.

(3) To return to the Method Screen Display, press the RESET key and hold it down while pressing the METH key. If the current Method has not been stores, the prompt will read

CLEAR CURRENT METHOD (Y/N)?

If it has been stored, the prompt will read

ENTER NUMBER OF METHOD

(4a) Press N to return to the Method you were working on before you called the directory.

Note: If the Method on the screen was not stored before calling the Directory, it will be erased if Y is entered in response to the CLEAR CURRENT METHOD (Y/N)? prompt. To preserve the Method, answer N.

(4b) Press Y to choose a different stored Method. The prompt will request

ENTER NUMBER OF METHOD

Type in the storage number of the Method to be displayed and press the ENTER key.

4.9. UNTIMED ISOCRATIC ANALYSIS

Untimed isocratic analyses are used primarily for methods development and optimization when you simply want the pump to run continuously at a constant flow rate and percent composition as you inject the sample and observe the results. An isocratic method of this type may not be stored or included in a Method Chain. This mode of operation permits modification of the parameters such as the flow rate and percentage of strong solvent at any time during the analysis by stepping to the Equilibration sector of the Method.

When the Series 4 is turned on, the Directory Screen display will appear and the prompt line will read:

POWER FAILURE ENTER PASSWORD

Enter a 2 character password or default the password entry by pressing the ENTER key.

```
                              DIRECTORY

        METHOD NUMBER                    METHOD DESCRIPTION


POWER FAILURE ENTER PASSWORD ■
```

Figure 4-8 - Empty Directory Screen (Screen Display Seen When Power is First Turned On)

```
METHOD   GEN                                                    TIME      MINS
SECTOR    TIME   FLOW
                 MLS CURVE     A%        B%        C%        D%        CURVE

EQUIL
  1
  2
  3
  4
  5
READY TIME            PMIN        MPa   PMAX        MPa   TEMPERATURE


ENTER METHOD DESCRIPTION ■
```

Figure 4-9 - Method Generation Screen Before Any Parameters are Entered

4.9.1. Generating An Untimed Isocratic Method:

(1) Press the RESET key and hold it down while pressing the GEN key on the Control Module. The prompt line will say:

PRESS METHOD, CHAIN, or CE.

Press the METH key.

The prompt will say

CLEAR CURRENT METHOD (Y/N)?

(2) Type Y to obtain the Method Generation Screen display.

(3) The prompt line on the Screen Display will read:

ENTER METHOD DESCRIPTION

You are now ready to enter the Method parameters. Enter or default the parameters as desired (see Untimed Isocratic Method Parameter Entries, below).

When all parameters entries have been made, the Series 4 will leave the Method Generation Mode automatically.

This is indicated by the cursor beginning to scan to update the Status Section of the screen page.

4.9.2. Parameter Entries For Untimed Isocratic Method

1. METHOD DESCRIPTION

The entry may be defaulted by pressing the ENTER key. To enter a Method description, type it in and press the ENTER key. Up to 40 alpha-numeric characters may be entered. When the entry is completely typed in, press the ENTER key to step to the next parameter.

Note: If the Method Description is defaulted on more than one Method, or if the same description is used for two Methods, when you attempt to store the second Method, the terminal will beep and the prompt will read:

DUPLICATE METHOD NAME, PRESS Y TO CHANGE OR ENTER TO CONTINUE.

Type Y to give the Method a different description. Press the ENTER key to leave the duplicate description.

Use the SHIFT and left and right arrow keys to edit the Method description if desired.

2. SOLVENT RESERVOIRS

Enter an identifying name for each solvent reservoir to be used in the Method. A reservoir must be identified in order to use it in the Method. Up to 5 alpha-numeric characters may be used. The prompt will say:

ENTER SOLVENT NAME FOR RESERVOIR A.

Enter a name for this reservoir (e.g., H20, ACN, THF) if it is to be used. Press the ENTER key to default the entry if this reservoir is not to be used.

Each of the 4 reservoirs should either be identified or defaulted. At least one of the reservoirs must be identified. If you attempt to default all 4 reservoirs identifications, the cursor will return to the Reservoir A identification position.

For a two-solvent mixture you could identify and use 2 reservoirs; e.g., A and B. Or, if the ideal solvent pair is unknown, you might use Reservoir A for acetonitrile, Reservoir B for methylene, Reservoir C for THF (tetrahydrofuran) and Reservoir D for water. Thus the Reservoir identification would be as follows:

ACN	MEOH	THF	H20
A%	B%	C%	D%

Since the parameters may be modified easily by stepping to the EQUIL Sector during an isocratic run, the actual reservoirs being used at any given time may be controlled with the percent composition.

> Note: To obtain increased capability or to run samples longer, 2 of the reservoirs can be filled with the same solvent. For example, A and C can both be filled with ACN and B and D both filled with H_2O. This increased volume capability for one solvent pair gives up to 66 hours of continuous run time at 1 ml/min. See 4.16 for further information.

3. EQUIL TIME

The default value for Equilibration time is 10. Press the ENTER key to enter an EQUIL time of 10. To enter a different equilibration time, type in a number between 0.1 and 999.9 minutes and press the ENTER key.

When the untimed isocratic analysis is started, the system will equilibrate for the time entered and then the "Ready" light on the Pump Module will light.

> Note: In the Untimed Isocratic Analysis mode, after the initial EQUIL time has expired, the "Ready" light is always lit, and the "Run" button is disabled.

4. FLOW RATE (MLS)

Type in a flow rate in ml/min. and press the ENTER key or merely press the ENTER key to obtain the default flow rate of 2 ml/min. The entry may be any number between 0.1 and 9.9 ml/min.

5. % SOLVENT

Type the percentage of solvent to be used and press the ENTER key for each reservoir except the last identified in step 2 unless all identified reservoirs will not be used. The program will automatically calculate the percentage for the last reservoir to be used so that the sum of the percentages will always equal 100%.

For example, if only two reservoirs were identified in step 2, when 60% is entered for the first reservoir, 40% will automatically be entered for the second reservoir. If four reservoirs were identified in step 2, but only 2 are to be used, the percentages for both reservoirs must be entered. If solvent is to be used from 4 identified reservoirs, then upon entry of the third percentage, the system will calculate the remaining percentage to make the percentages total 100%.

Note: If the user attempts to enter the final % solvent, the value entered automatically by the Series 4, the numbers entered will appear as a TIME entry in the following sector. If this happens, use the shift and left arrow keys to return to the beginning of the parameter entry line, and use the CE key to remove the improper value. Type in the correct value for the Sector 1 Time (zero for an untimed isocratic analysis) and press the ENTER key.

If the user attempts to enter percentages totaling more than 100%, the Series 4 will beep and the prompt:

ERROR, CE
or
TOTAL PERCENT EXCEEDS 100%

will appear. Re-enter the proper percentages.

6. SECTOR 1 TIME

In the untimed isocratic mode, Sectors 1-5 are not used since the pump runs continuously. Therefore, press the ENTER key to obtain the default value of 0 for T1. This indicates to the Series 4 that this Method is for an untimed isocratic analysis.

7. READY TIME

Defaulting the Sector 1 Time entry by pressing the ENTER key causes the Series 4 to automatically enter the default Ready Time of INF, or infinity. Entering 0 will also give INFinite running time.

Note: A Method with INF Ready time cannot be used in a chain.

Note: Ready Time is the amount of time that the system will remain "Ready" and waiting to run without automatically shutting down. Since in the Untimed Isocratic mode, after the initial equilibration time has expired the pump begins to run and runs continuously until it is stopped manually, "Ready Time" is equal to infinity. Also, since the system remains ready once the equilibration times out, and since the pump is running continuously, the READY/RUN button is disabled in this mode.

8. PMIN

Type in a value for the minimum pressure and press the ENTER key, or default this parameter by pressing the ENTER key. A reasonable value for PMIN would be 0.5-2.0. Permissible values are 0-41.9 MPa. If the ENTER key is pressed to default the entry, the default value for PMIN will be 0. The PMIN entered must be less than the PMAX entry (see below).

Note: If the system does not reach PMIN +10% within one minute, the system will assume that a leak exists or that the reservoir has run out of solvent and the pump will automatically shut down. Thus a value for PMIN greater than zero should be entered for safety if the System is to run unattended.

9. PMAX

Default this parameter by pressing the ENTER key or enter a value for the maximum pressure which will protect the column(s) used in the analysis from excessive pressure. The default value for this entry is 42 MPa (6000 psi). 42 MPa is normally used unless the column is fragile. Any value betweeen 0.1 and 42 MPa may be entered for PMAX. If a value is entered for PMAX which is less than or equal to the value entered for PMIN, the LC Terminal will beep, and the prompt will ask for a new value for PMAX. If the value for PMAX is reached, the system will automatically shut down.

10. TEMPERATURE

If the optional column oven is not used, the Series 4 will skip this parameter. If the oven is connected, the entry should be defaulted or an entry should be made. The default value for temperature is OFF. If the column oven is to be used, enter a value between 35°C temperature and 99°C. If the value entered is less than 35°C, the program will default to a power OFF condition.

If a heated oven is to be used with an untimed isocratic run, there is no automatic cool-down sequence to protect the column. The operator must modify the Method without stopping the pumps to turn off the oven and then must allow the solvent to continue to flow for a sufficient time to cool down the column.

To provide a cool-down sequence to protect the column after running in the untimed isocratic mode:

(1) Press the RESET key and hold it down while pressing the EQUIL key. This will step to the equilibration sector.

CAUTION: DO NOT STEP TO THE SHUTDOWN SECTOR TO MODIFY THE TEMPERATURE

(2) Press the RESET key and hold it down while pressing the MODIFY key.

(3) Press the TEMP parameter key to step directly to the temperature parameter.

(4) Type 10* and press the ENTER key. This will enter 10°C, which will turn the oven OFF.

(5) Allow the solvent to flow for a sufficient period of time to cool the column or remove the column from the oven before using the SOTP key to stop the pump.

*Any value between 10 and 34 may be used.

A Sample Screen Display for an Untimed Isocratic Analysis after parameter entries have been made complete is shown in Figure 4-10.

```
METHOD   UNTIMED ISOCRATIC ANALYSIS                          TIME 10.0 MINS

SECTOR    TIME    FLOW        ACN       H2O
                  MLS CURVE   A%        B%        C%        D%        CURVE

EQUIL     10.0    2.0         80.0      20.0      0.0       0.0
  1        0.0
  2
  3
  4
  5
READY TIME    INF     PMIN     0.0 MPa  PMAX   42.0 MPa  TEMPERATURE OFF


VIAL    INJECTION          TOTAL              CURRENT              PRESSURE
          NO.              TIME               SECTOR     TIME      MPa
                            0.0               SHTDN       0.0      0.0

                 FLOW       ACN       H2O

CURRENT VALUES   0.0        0.0       0.0
```

Figure 4-10 - A Sample Untimed Isocratic Method Screen Display after all parameters have been entered

Notice that the screen display is divided into 3 sections: The Current Method Section, the Current Status Section and the line for prompts, queries and error messages.

The Current Method is always shown at the top of the screen unless the Method screen display is temporarily replaced by the Chain display or the Directory because of an operator request.

The Current Status Section appears when Method Operation or Modification is complete and is continuously and automatically updated so that the operator can see at a glance what is happening.

Operation prompts appear on the last line of the display.

4.9.3. Storing the Untimed Isocratic Method

(1) Press the RESET key and hold it down while pressing the STORE key. the prompt will request:

ENTER METHOD STORAGE NUMBER

(2a) Press the ENTER key to store the Method under the next available storage number; or

(2b) Type in a number (1-9) and press the ENTER key.

If you have entered a number which has already been used to store a Method or if no Method storage number is available, the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

will appear.

(3a) To replace the existing Method in memory with the currently-displayed Method type Y and press the ENTER key.

(3b) To avoid erasing the existing Method from memory, type N and press the ENTER key. This will exit from the STORE routine.

(4) Repeat steps (1) and (2a) or (2b) above, entering a number which has not previously been assigned to a stored Method.

(5) Check the Method description line of the Screen Page to confirm the storage number for the Method.

4.9.4. Running An Untimed Isocratic Method

The Method to be run must be displayed on the Control Module screen.

Note: Always check the current values displayed on the bottom portion of the screen against the Method at the top of the screen before starting a new Method to make sure that an abrupt or improper solvent change which might damage the column will not be made by the Series 4.

To start the pump, on the Control Module press the RESET key and hold it down while pressing the START key.

This will start the pump. Following the expiration of the EQUIL time, the READY light on the pump Module will illuminate and will remain on until the program is stopped. In the untimed isocratic mode the RUN button is disabled.

4.9.5. Stopping An Untimed Isocratic Method

Press the RESET key and hold it down while pressing the STOP key.

> **Note:** In the Untimed Isocratic analysis mode the pump will run continuously until it is stopped with the STOP key or until the PMAX value is exceeded, or, if a value greater than zero has been entered for PMIN, until the solvent supply is exhausted.

> **CAUTION:** If a heated oven is to be used with an untimed isocratic run, there is no automatic cool-down sequence to protect the column. The operator must modify the Method without stopping the pumps to turn off the oven and then must allow the solvent to continue to flow for a sufficient time to cool down the column.

To provide a cool-down sequence to protect the column after running in the untimed isocratic mode:

(1) Press the RESET key and hold it down while pressing the EQUIL key. This will step to the equilibration sector.

> **CAUTION:** DO NOT STEP TO THE SHUTDOWN SECTOR TO MODIFY THE TEMPERATURE

(2) Press the RESET Key and hold it down while pressing the MODIFY key.

(3) Press the TEMP parameter key to step directly to the temperature parameter.

(4) Type 10* and press the ENTER key. This will enter 10°C, which will turn the oven OFF.

(5) Allow the solvent to flow for a sufficient period of time to cool the column or remove the column from the oven before using the STOP key to stop the pump.

4.9.6. Stepping To The EQUIL Sector

In this mode the Method may be modified at any time by stepping to the EQUIL sector.

To step to the EQUIL sector, press the RESET key and hold it down while pressing the blue EQUIL key (do not confuse the blue EQUIL key with the white TEQ parameter entry key). The program will step immediately to the EQUIL sector.

*Any value between 10 and 34 may be used.

Note: If you attempt to modify a Method while the program is in READY or RUN, the Control Module will beep and the prompt will say:

MODIFY METHOD IN EQUIL OR SHUTDOWN ONLY

4.9.7. Modifying An Untimed Isocratic Method

(1) The Method to be modified must be displayed on the screen.

(2) Press the RESET key and hold it down while pressing the MOD key.

(3a) Press the RESET key and hold it down while pressing the appropriate parameter key (e.g., TEQ). The Series 4 will step directly to this data entry position.

or

(3b) Press the ENTER Key to tell the Series 4 to step through the parameter entries. After the ENTER key has been pressed once, the program will step to the Method Description data entry position and the prompt will read:

ENTER METHOD DESCRIPTION

(4) Enter a Method Description (up to 40 alpha-numeric characters may be used), or default this parameter by pressing the ENTER key. The cursor will step to the next data entry position.

Note: It is possible to modify reservoir identifications but not to identify additional reservoirs. To identify additional reservoirs, a new Method must be generated.

(5) Use the ENTER key to step through the parameters, making changes as necessary.

You must step through all the parameters to exit from MODIFY. Just press the ENTER key to pass any parameter which does not need modification or hold down the REPEAT and ENTER keys to get out of the Modification mode.

Note: Once you have entered the Modify mode, you can only escape by pushing the CE key before making any modifications to the Method. Otherwise you must step through all the parameters to exit before attempting to run or store the Method. This is easily accomplished by using the ENTER key in conjunction with the REPEAT key. If nothing happens when you try to store or run a Method, check to see whether the System is still in the Modify mode.

4.10. TIMED ISOCRATIC ANALYSIS

Once a method has been developed and the optimum parameters to produce a satisfactory analysis for that sample type are known, the user can store the parameters, or Method for that analysis so that it may be used in a chain, along with gradient methods if desired, for unattended isocratic analyses. The internal clock on the Series 4 is used to control analysis time.

The 4 solvents can be employed simultaneously to produce unique isocratic mobile phase combinations which can produce separations not achievable with simple binary mixtures and which can replace gradient separations and eliminate the equilibration time and accelerated column wear which accompany gradient operation.

A Timed Isocratic Method is similar to an Untimed Isocratic Method except that the analysis time is controlled by the Series 4 internal clocks. The Timed Isocratic Method may either be generated as a new Method, or an existing Untimed Isocratic Method may be modified to make it a Timed Isocratic Method. Figure 4-11 shows the Method Screen Display for a Timed Isocratic Analysis.

```
METHOD   TIMED ISOCRATIC METHOD                              TIME 25.0 MINS

SECTOR     TIME     FLOW          ACN        H2O
                    MLS CURVE     A%         B%          C%          D%          CURVE

EQUIL      10.0     2.0           60.0       40.0
  1        15.0     2.0           60.0       40.0
  2         0.0
  3
  4
  5
READY TIME        60.0  PMIN   0.0   MPa  PMAX   42.0 MPa  TEMPERATURE OFF


VIAL     INJECTION          TOTAL                CURRENT                 PRESSURE
           NO.              TIME          SECTOR        TIME              MPa
                             0.0          SHTDN         0.0               0.0

■                  FLOW          ACN        H2O

CURRENT VALUES     0.0           0.0        0.0
```

Figure 4-11 - A Sample Timed Isocratic Method Screen Display after parameters have been entered

4.10.1 Modifying An Existing Method

The steps for modifying a Method to create a Timed Isocratic Method are the same as those given for modifying an Untimed Isocratic Method, except that there is a Sector 1 TIME entry and the READY TIME parameter entry is different.

The Method to be modified must be displayed on the screen.

(1) Press the RESET key and hold it down while pressing the MOD key.

The Series 4 enters the MODIFY mode. The prompt

SELECT SECTOR OR PRESS ENTER KEY

will appear.

(2a) To step directly to a particular parameter, press the appropriate parameter key (e.g., TEQ).

or

(2b) Press the ENTER key once to step through the parameters. The program will now be in the Method Description data entry position.

See below for instructions on parameter entry for a Timed Isocratic Method.

> **Note:** It is not possible to identify additional reservoirs by modifying a Method.

When all parameter entries have been made, the Series 4 will leave the MODIFY mode automatically. The terminal will beep and the prompt:

REMEMBER TO STORE MODIFIED METHOD

will appear.

> **Note:** Once you have entered the Modify mode, you can only escape by pressing the CE key before making any modifications to the Method. Once modification has begun, you must step through all the parameters to exit from the Modify Mode. This can be done quickly by holding down the REPEAT and ENTER keys simultaneously. If a Method will not run, check to see whether the Method is in the Modify mode. This will be indicated by the word "Modify" on the first line of the screen display next to the Method Description.

4.10.2. Storing The Modified Method

(1) Press the RESET Key and hold it down while pressing the STORE key. The prompt will request:

ENTER METHOD STORAGE NUMBER

(2a) Press the ENTER key to store the Method under the next available storage number; or

(2b) Type in a number (1-9) and press the ENTER key.

If you have entered a number which has already been used to store a Method, the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

will appear.

(3a) To replace the existing Method in memory with the currently-displayed Method type Y and press the ENTER key.

(3b) To avoid erasing the existing Method from memory, type N and press the ENTER key. This will exit from the STORE routine.

(4) Repeat steps (1) and (2a) or (2b) above, entering a number which has not previously been assigned to a stored Method.

(5) Check the Method Description line of the Screen Page to confirm the storage number for the Method.

If The Method which has been modified has been used in a chain, the prompt:

METHOD USED IN CHAIN, OVERWRITE (Y/N)?

will appear.

To preserve the unmodified method, type N. This exits from the STORE routine.

Answering Y will overwrite the original method.

4.10.3. Generating A Timed Isocratic Method

The steps for generating a timed isocratic Method are the same as those for generating an untimed isocratic Method except that there is a Sector 1 TIME entry and the READY TIME parameter entry is different.

(1) Press the RESET key and hold it down while pressing the GEN key. The prompt line will say:

PRESS METHOD, CHAIN, OR CE

(2) Press the METH key. The prompt:

CLEAR CURRENT METHOD (Y/N)?

will appear.

(3) Type Y. The Screen will clear and the Method Generation Screen Display will appear. The prompt line on the Screen Display will read:

ENTER METHOD DESCRIPTION

You are now ready to enter the Method parameters. Enter or default the parameters as desired (see Timed Isocratic Method Parameter Entries, below).

When all parameter entries have been made, the Series 4 will leave the Method Generation mode automatically.

4.10.4. Parameter Entry For A Timed Isocratic Method

1. METHOD DESCRIPTION

   Type in a description of the method, e.g., "VITAMIN A ASSAY". Up to 40 alpha-numeric characters may be entered. When the entry is completely typed in, press the ENTER key to step to the next parameter.

   If the same description has been used for a previously-stored Method, the prompt will read:

DUPLICATE METHOD NAME. PRESS Y TO CHANGE OR ENTER TO CONTINUE

   Type Y to rename the Method. Press the ENTER key to preserve the duplicate descriptions.

2. SOLVENT RESERVOIRS

   The next data entry position is for identification of solvent Reservoir A. Enter a name for this reservoir (e.g., H20, ACN, THF) if it is to be used. Default the entry by pressing the ENTER key if this reservoir is not to be used. Enter an identification for each solvent reservoir to be used. A reservoir must be identified in order to use it in the method. Up to 5 alpha-numeric characters may be used.

   For example, for a two-solvent mixture you could identify and use reservoirs A and B. If all four reservoirs are identified, they do not necessarily have to be used, but unless they have been identified they cannot be used. The actual use is governed by the entries of percent composition, as in the example below.

ACN	MEOH	THF	H20
A%	B%	C%	D%
50	0	0	50

   Note: To obtain increased capability or to run samples longer when the system is unattended, 2 of the resevoirs can be filled with the same solvent. For example, A and C can both be filled with ACN and B and D both filled with H_2O. This increased volume capability for one solvent pair gives up to 66 hours of unattended run time at 1 ml/min. See Section 4.16 for details.

EQUILIBRATION SECTOR

3. EQUIL TIME

Type in an equilibration time and press the ENTER key or merely press the ENTER key to obtain the Default Value of 10 min. The equilibration time should be long enough to allow the column to equilibrate and for the temperature to equilibrate if the optional column oven is to be used. Permissible values are 0.1 to 999.9 minutes. The system will not become "Ready" to run the Method until the equilibration time entered has passed.

> **Note:** If the Method is used in a chain where a solvent change necessitates a longer equilibration time, the longer equilibration time should be added to the Method which follows the solvent change.

4. EQUILIBRATION FLOW RATE (MLS)

Type in a flow rate in ml/min and press the ENTER key or merely press the ENTER key to obtain the default flow rate of 2 ml/min. The entry may be any value between 0.1 and 9.9.

5. % SOLVENT

Type in the percent of solvent to be used from each reservoir identified in step 2 and press the ENTER key. The program will automatically calculate the percentage for the last reservoir to be used so that the sum of the percentages will always equal 100%.

For example, if only two reservoirs were identified in step 2, when 60% is entered for the first reservoir, 40% will automatically be entered for the second reservoir. If four reservoirs were identified in step 2, but only two are to be used, the percentages for both reservoirs must be entered. If solvent is to be used from 4 identified reservoirs, then upon entry of the third percentage, the system will calculate the remaining percentage to make the percentages total 100%.

If the user attempts to enter percentages totaling more than 100%, the Series 4 will beep and the prompt:

ERROR, CE
or
TOTAL PERCENT EXCEEDS 100%

will appear. Re-enter the proper percentages.

SECTOR 1 ENTRIES

A Timed Isocratic Analysis uses the EQUIL Sector for Equilibration and Sector 1 for the analysis. Normally the only entry in Sector 1 which will differ from the EQUIL Sector entries is the TIME entry.

6. TIME 1

Type in a time for the isocratic analysis and press the ENTER key. Typical times are 0.5 to 1.0 minutes longer than the time required to elute the last peak. Permissible values are 0.1 to 999.9 minutes.

T1 must be greater than zero to give a Timed Isocratic Method which can be run and stored. If zero is entered, the READY TIME will be infinite.

7. FLOW RATE (MLS)

Type in a flow rate in ml/min. and press the ENTER key or just press the ENTER key to obtain the default flow rate of 2 ml/min. The entry may be any number between 0.1 and 9.9. The same flow rate is used in Sector 1 as was used in the EQUIL Sector. (For a Flow Program, see Section 4.12.)

8. % SOLVENT

Type in the percent solvent and press the ENTER key for each reservoir identified in step 2, above. For an isocratic analysis the % SOLVENT entries made in Sector 1 should be the same as those entered in the EQUIL Sector.

SECTOR 2 ENTRIES

Press the ENTER key to obtain the default value of 0 for Sector 2 Time. (Note that the default value for EQUIL TIME is 10 minutes, but the default value for time in sectors 1-4 is zero.)

The cursor should now be at the READY TIME entry position.

9. READY TIME

Type in a READY TIME and press the ENTER key or default the entry by pressing the ENTER key. Permissible values are 0.1 to 999.9. The default value in the timed isocratic mode is 60 min. "Ready Time" is the amount of time the system will remain "ready" and waiting to run without shutting down.

Note: A Ready Time of 0 minutes or infinite time must NOT be used since entering zero will automatically change the program to the untimed isocratic mode and erase Sector 1 entries. A Method with infinite Ready Time or zero may not be used in a Method Chain.

10. PMIN

Type in a value for a minimum pressure and press the ENTER key or default the entry by pressing the ENTER key. If the Series 4 is to run unattended, it is advisable to enter a PMIN so that the system will shut down if a leak exists or if the reservoir runs out of solvent. The default value for PMIN is zero (0). Typical values are 0 to 2 MPa. Permissible values are 0.0 to 41.9 MPa. The PMIN entered must be less than the PMAX entry (see below).

Note: If the system does not reach PMIN +10% within one minute, the Series 4 will assume that a leak exists or that the reservoir has run out of solvent, and the pump will automatically shut down.

11. PMAX

Type in a value for the maximum pressure which will protect the column(s) used in the analysis from excessive pressure, or default the parameter by pressing the ENTER key. If the value for PMAX is reached, the system will automatically shut down.

Any value between 0.1 and 42 MPa may be entered for PMAX. The default value for this entry is 42 MPa (6000 psi) and is normally used unless the column is fragile.

If a value is entered for PMAX which is less than or equal to the value entered for PMIN, the Control Module will beep, and the prompt will ask for a new value for PMAX.

12. TEMPERATURE

If the optional column oven is connected, type in a value between 35°C and 99°C and press the ENTER key or default the parameter by pressing the ENTER key. The default value for temperature is OFF. If the value entered is below 35°C the system will automatically default to a power OFF condition.

If the column oven is not connected to the system, the Series 4 will automatically skip this entry.

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The Cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

The parameter entries are now complete. The terminal will beep and the prompt will remind the user to store the Method.

4.10.5. Storing A Timed Isocratic Method

(1) Check the screen display to see that the required parameters have been entered correctly.

(2) Press the RESET key and hold it down while pressing the STORE key.

(3) The screen prompt will request:

ENTER METHOD STORAGE NUMBER

(4a) The user can enter a number from 1 to 9 or press the ENTER key to cause the system to assign the next available number to the method to be stored.

(4b) Entering a number which has previously been used to store a Method will cause the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

to appear.

(5a) Answering N will exit from the STORE program. Go back to step 2 and repeat the Store procedure, assigning a different storage number.

(5b) Answering Y will replace the method previoulsy stored under that number with the method presently shown on the screen display.

(5c) If a modified method which has been used in a chain is to be stored, the prompt:

METHOD EXISTS IN CHAIN, OVERWRITE (Y/N)?

will appear.

To preserve the unmodified method, type N.

Answering N will exit from the STORE program. Go back to step 2 and repeat the STORE procedure, assigning a different storage number.

Answering Y wil overwrite the original method. Make sure that the modifications are appropriate for use in the Chain.

4.10.6. Recalling A Stored Method To The Screen

(1) Recall the stored Method to the screen by pressing the RESET key and holding it down while pressing the METH key. The prompt will ask for the number of the Method to be recalled.

(2) Type in the Method number to the screen from storage and press the ENTER key.

4.10.7. Running The Timed Isocratic Analysis

The Method to be run must be displayed. If another Method is currently displayed, see the instructions for recalling a method from storage (see Section 4.10.6.) or generate the Method.

Note: Always check the current values displayed on the bottom portion of the screen against the Method at the top of the screen before starting a new Method to make sure that an abrupt or improper solvent change might damage the column will not be made by the Series 4.

(1) To start the pump, press the RESET key and hold it down while pressing the START key.

The pump will start and the system will equilibrate for the time entered under EQUIL TIME. When the equilibration time has passed, the system will become "Ready" and the READY light on the Pump Module will light.

(2) Inject the sample.

(3) Immediately press the RUN button on the Pump Module.

The pump will run for the time entered in Sector 1 and then the system will automatically go back to the equilibration sector. When the equilibration times out, the READY light illuminates on the Pump Module.

If the RUN button on the Pump Module is pressed, the pump will run for the time entered in Sector 1, automatically return to the equilibration sector and, when equilibration times out, the Series 4 becomes "Ready" again.

If the RUN button on the Pump Module is not pressed, the system will remain "Ready" and the pump will run for the time entered as READY TIME. Then the Series 4 will shut down.

4.10.8. Stepping To The EQUIL Sector

In the Timed Isocratic Analysis mode, the Method may be modified before the run is initiated, and while the pump is in the "EQUIL" sector.

The Method to be modified must be displayed on the Control Module. If it is not, call the Method to the screen before continuing with the instructions below.

If the System is not in the SHUTDOWN or in EQUIL sector, press the RESET key and hold it down while pressing the blue EQUIL key. The program will step immediately to EQUIL.

4.10.9. Modifying A Timed Isocratic Analysis Method

(1) To modify the Method, press the RESET key and hold it down while pressing the MOD key.

(2) Use the ENTER key to step through the parameters, making changes as necessary; or, press the RESET key and the appropriate parameter key; e.g., T1, T5, etc.) to step directly to the parameter to be changed.

> **Note:** If you inadvertently pass a data entry position, use the shift key and the left arrow key to return to the beginning of the line which contains the data entry position. Use the ENTER key to step across the line to the proper data entry position.

4.10.10. Stopping A Timed Isocratic Analysis

Press the RESET key and hold it down while pressing the STOP key. This will abort an analysis in progess

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

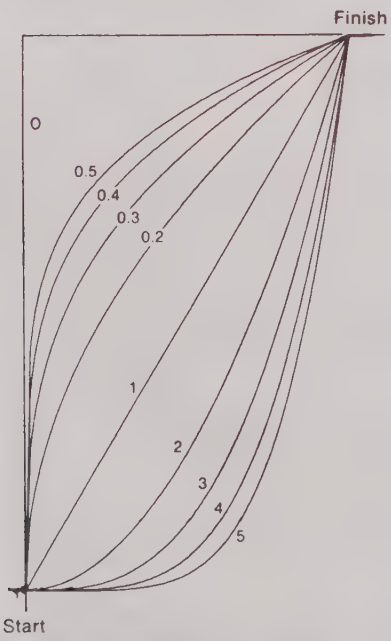
4.10.11. Using A Timed Isocratic Method In A Chain

See Section 4.14 for instructions on Chaining Methods for unattended analysis.

4.11. GRADIENT ANALYSIS

4.11.1. Introduction To Gradient Analysis

If a change in the elution strength of the mobile phase with time is necessary to obtain a satisfactory chromatogram, a gradient program is used. In a simple linear gradient program (curve 1) the gradient is produced by the gradual increase in percentage of the stronger solvent and simultaneous reduction of the weaker solvent while a constant flow rate is maintained*. Concave gradients (curves 2, 3, 4 and 5) are used to maintain early resolution and sweep off later components. Convex gradients (curves 0.2, 0.3, 0.4 and 0.5) are used to speed up the early peaks and maintain resolution of the later peaks. The linear and concave gradients are the most useful in LC. Figure 4-12 shows the gradient curves available on the Series 4.



Note: *Because fractional entries cannot be made on the Series 4, 0.2, 0.3, 0.4 and 0.5 are used by convention to indicate convex gradients.*

Figure 4-12 - Gradient Curves Available on the Series 4

***Note:** It is possible to combine a gradient program with a flow program on the Series 4. This is discussed in Section 4.13.

4.11.2. Generating A Gradient Method

It is possible to modify an existing Method to create a Gradient Method, but normally a new Gradient Method would be generated.

4.11.2.1. MODIFYING AN EXISTING METHOD

The Method to be modified must be displayed on the Control Module and must be in SHUTDOWN or EQUIL. Press the RESET key and hold it down while pressing the MOD key. The prompt

SELECT SECTOR OR PRESS ENTER KEY

will appear. Press the appropriate parameter key or press the ENTER key to step through the parameters. See below for instructions on parameter entry for a Gradient Method.

When all parameter entries have been made, the Series 4 will leave the MODIFY mode automatically. The terminal will beep and the prompt will remind the user to store the Method.

4.11.2.2. GENERATING A GRADIENT METHOD

(1) Press the RESET key and hold it down while pressing the GEN key on the Control Module. The prompt line will say:

PRESS METHOD, CHAIN, OR CE

(2) Press the METH key. The prompt:

CLEAR CURRENT METHOD (Y/N)?

will appear.

(3) Type Y. The screen will clear and the Method Generation Screen Display will appear. The prompt line on the Screen Display will read:

ENTER METHOD DESCRIPTION

You are now ready to enter the Gradient Method parameters. enter or default the parameters as desired (see Gradient Analysis Parameter Entries, below).

When all parameter entries have been made, the Series 4 will leave the Method Generation mode automatically.

Figure 4-13 shows the Method Screen Display for a linear Gradient Analysis. Figure 4-14 shows the Method Screen Display for a concave Gradient Analysis.

The current Method is always shown at the top of the screen unless it is temporarily replaced by the Chain display or the Directory because of an operator request.

```
METHOD   LINEAR GRADIENT                                        TIME 22.0 MINS

SECTOR    TIME    FLOW      ACN     H2O
                  MLS CURVE A%      B%          C%        D%        CURVE

EQUIL     10.0    2.0       20.0    80.0
  1        5.0    2.0       80.0    20.0                            1
  2        5.0    2.0       80.0    20.0                            0
  3        2.0    2.0       20.0    80.0                            1
  4        0.0
  5
READY TIME      60.0   PMIN  0.0   MPa   PMAX  42.0  MPa  TEMPERATURE OFF

VIAL     INJECTION          TOTAL                CURRENT              PRESSURE
           NO.              TIME          SECTOR       TIME           MPa
                            0.0           SHTDN        0.0            0.0

                 FLOW       ACN     H2O

CURRENT VALUES   0.0        20.0    80.0
```

Figure 4-13 - A Sample Screen Display for a Linear Gradient Analysis

```
METHOD   CONCAVE GRADIENT                                        TIME  3.4 MINS

SECTOR    TIME    FLOW      ACN       MEOH      P04       B-6
                  MLS CURVE A%        B%        C%        D%        CURVE

EQUIL     1.3     3.0       38.0      10.0      3.0       49.0
  1       2.1     3.0       58.0      10.0      3.0       29.0        2
  2       0.0
  3
  4
  5
READY TIME      60.0   PMIN  18.0  MPa   PMAX  42.0 MPa  TEMPERATURE 40

VIAL     INJECTION          TOTAL                CURRENT              PRESSURE
           NO.              TIME          SECTOR       TIME           MPa
                            1.4           READY        0.0            21.8

                 FLOW       ACN       MEOH      P04       B-6

CURRENT VALUES   3.0        38.0      10.0      3.0       49.0
```

Figure 4-14 - A Sample Screen Display for a Concave Gradient Analysis

4.11.3. Parameter Entry For Gradient Analysis

1. METHOD DESCRIPTION

Type in a description of the method and press the ENTER key or default the entry by pressing the ENTER key to step to the next parameter. Up to 40 alpha-numeric characters may be used.

2. SOLVENT RESERVOIRS

The cursor will step to the first reservoir identification position (Reservoir A). Enter an identification for this reservoir (e.g., H2O, ACN, MEOH, THF) if it is to be used. Up to 5 alpha-numeric characters may be entered. Default the entry by pressing the ENTER key if a reservoir is not to be used. A reservoir must be identified in order to use it in the Method. Each of the 4 reservoirs should either be identified or defaulted.

For a two-solvent mixture, you would identify and use two reservoirs, A and B. For a four solvent mixture, all four reservoirs must be identified.

> **Note:** To obtain increased capability 2 of each of the reservoirs can be filled with the same solvent. For example, A and C can both be filled with ACN and B and D both filled with H2O. This increased volume capability for one solvent pair gives up to 66 hours of run time at 1 ml/min. See Section 4.16 for details.

3. EQUIL TIME

Type in an equilibration time and press the ENTER key or default this entry by pressing the ENTER key. The default value is 10 min. The equilibration time should be long enough to allow the column to equilibrate with the new solvent (at least 10 column volumes should be used) and for the temperature to equilibrate if the column oven is to be used. Permissible values are 0.1 to 999.9 minutes. The system will not become "Ready" until the equilibration time entered has passed.

> Note: If the Method is used in a chain where a solvent change necessitates a longer equilibration time, the longer equilibration time should be added to the method which follows the solvent change.

EQUILIBRATION ENTRIES

4. FLOW RATE (MLS)

Type in a flow rate in ml/min. and press the ENTER key or just press the ENTER key to obtain the default flow rate of 2 ml/min. This entry may be any number between 0.1 and 9.9.

5. % SOLVENT

The % Solvent data entry positions are for the percentage of solvent to be taken from each of the reservoirs identified in step 2, above. Type in the percent solvent to be used from each identified reservoir except the last one and press the ENTER key. The system should be equilibrated with the solvent to be used at the beginning of the gradient. The system will automatically calculate the percentage of solvent to be taken from the last identified reservoir so that the sum of the percentages will equal 100%.

For example, if only two reservoirs were identified in step 2, when 60% is entered for the first reservoir, the system will automatically enter 40% for the second reservoir. If four reservoirs were identified in step 2 but only two are to be used in the method, the percentages for both must be entered by the user. If the solvent is to be used from 4 identified reservoirs, upon entry of the third percentage, the system will calculate the fourth percentage.

If the user attemps to enter percentages totaling more than 100%, the Series 4 will beep and the prompt:

ERROR, CE
or
TOTAL PERCENT EXCEEDS 100%

will appear. Re-enter the proper percentages.

SECTOR ENTRIES

The Series 4 has five different independently-programmable time sectors. For each of these sectors, the user can specify time, flow rate and flow curve, if any (see Section 4.13), percent solvent from each solvent reservoir and the gradient curve. Two separate curves are allowed in each sector: one flow rate change and one percent composition change of two percent composition changes may be made in each sector (see the Operator Aids Section - How the Series 4 Counts Curves).

Figure 4-15 shows how the linear gradient shown in the Figure 4-13 example looks as a plot of percent strong solvent against time.

After the EQUIL entries are completed, the cursor will go to the Sector 1 TIME entry position, then to FLOW RATE (MLS) and FLOW CURVE (if the flow rate is changed), and through % SOLVENT for each identifified reservoir, to CURVE. The Sector 2 entries are then made, then Sector 3 entries, and so forth. Pressing the ENTER key when the cursor is in a SECTOR TIME entry position enters 0, which signifies to the Series 4 that no other sectors are to be used.

Steps 6 - 10 describe sector 1-5 parameter entries.

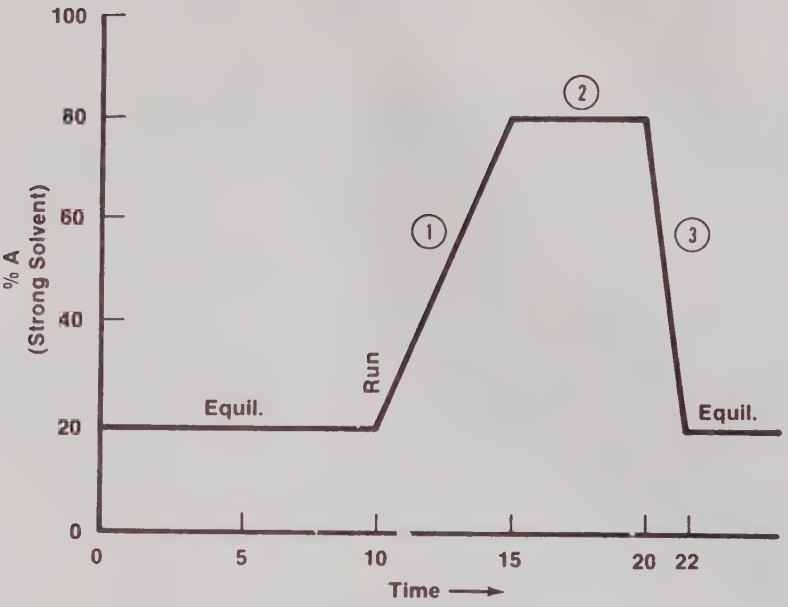


Figure 4-15 - The Linear Gradient Plotted as a Percentage of Strong Solvent Against Time.

SECTOR 1-5 ENTRIES

6. TIME

Type in the time over which the change in the mobile phase in this sector of the Method is to occur. Permissible values are 0.1 to 999.9 minutes.

If you refer to Figure 4-13 showing the example Linear Gradient Method screen display, you will see that 10 minutes has been entered for equilibration time, and that 5 minutes has been entered under TIME in Sector 1. During Equilibration the mobile phase is 80% water. The 5 minutes entered in Sector 1 will cause the change from 80% water to 80% acetronitrile to take 5 mintues.

7. FLOW RATE (MLS)

Type in a flow rate in ml/min. for this sector of the Method and press the ENTER key or press the ENTER key to obtain the default flow rate of 2 ml/min. This entry may be any value between 0.1 and 9.9.

8. FLOW RATE (CURVE)

If the flow rate entered in Sector 1 differs from that used in the EQUIL sector, or if the flow rate is changed in subsequent Sectors, the cursor will step to the FLOW CURVE position. Type in the number for the flow curve and press the ENTER key. A linear flow curve is 1. Convex flow curves are 0.2, 0.3, 0.4 and 0.5. Concave flow curves are 2, 3, 4 and 5. These curves are shown in Figure 4-16.*

Two separate curves are allowed in each sector: one flow rate curve and one solvent curve or two solvent curves. (See the Operator Aids Section - How The Series 4 Counts Curves.)

9. % SOLVENT

The cursor will step to the entry position for the first reservoir identified in step 2, above. Type in the percent solvent from that reservoir which should be present in the mobile phase at the end of the gradient and press the ENTER key. The cursor will step to the entry position for the next reservoir identified in step 2, above. Enter the percent solvent which should be present in the mobile phase at the end of the gradient and press the ENTER key. Repeat this for each reservoir identified.

10. CURVE

Type in the number for the gradient curve and press the ENTER key. A linear gradient curve is 1. Convex gradients are 0.2, 0.3, 0.4 and 0.5. Concave gradients are 2, 3, 4 and 5. Refer to Figure 4-12.

The Series 4 will now step to the next sector. Repeat the entries (6-10) for each sector to be used. After all sector entries have been made, enter 0 under time in the next sector. This indicates the end of the sector entries to the Series 4.

11. READY TIME

Type in a READY TIME and press the ENTER key or default the entry by pressing the ENTER key. Permissible values are 0.1 to 999.0. The default value is 60 min. Ready time is the amount of time the system will remain "ready" and waiting to run without shutting down.

> **Note:** A Ready Time of zero (0) minutes or infinite time must NOT be used since entering zero will automatically change the program to the untimed isocratic mode and erase all entries in sectors 1-5.

*Flow Curves are discussed in Section 4.12. Combining Flow curves with Gradient curves are discussed in Section 4.13.

12. PMIN

Type in a value for the minimum pressure and press the ENTER key or default the entry by pressing the ENTER key. The default value for PMIN is zero (0). Typical values are 0 to 2 MPa. Permissible values are 0.0 to 41.9 MPa. The PMIN entered must be less than the PMAX entry (see below).

> **Note:** If the system does not reach PMIN within one minute, the Series 4 will assume that a leak exists or that the reservoir has run out of solvent, and the pump will automatically shut down. It is therefore advisable to enter a value for PMIN which is greater than zero when the system is to run unattended.

13. PMAX

Type in a value for the maximum pressure which will protect the column(s) used in the analysis from excessive pressure or default the entry by pressing the ENTER key. If the value for PMAX is reached, the system will automatically shut down.

Any value between 0.1 and 42 MPa may be entered for PMAX. The default value for this entry is 42 MPa (6000 psi). This is normally used unless the column is fragile.

If a value is entered for PMAX which is less than or equal to the value entered for PMIN, the Control Module will beep, and the prompt will ask for a new value for PMAX.

14. TEMPERATURE

If the optional column oven is connected, type in a value between 35°C and 99°C and press the ENTER key or default the parameter by pressing the ENTER key. The default value is a power OFF condition. If the value entered is below 35°C, the system will automatically default to temperature control OFF.

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentially use the STOP key to stop the pump during the cool-down sequence.

The Gradient Method Generation is now complete. The terminal will beep and the prompt will remind the user to store the Method.

4.11.4. Storing A Gradient Analysis Method

(1) Check the screen display to see that the required parameters have been entered correctly.

(2) Press the RESET key and hold it down while pressing the STORE key.

(3) The screen prompt will request:

ENTER METHOD STORAGE NUMBER

(4a) The user may enter a number from 1 to 9 or press the ENTER key to cause the system to assign the next available number to the Method to be stored.

(4b) Entering a number which has previously been used to store a Method will cause the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

to appear.

(5a) Answering N will cause the system to exit from the STORE Program. Go back to step (2) and repeat the storage procedure, assigning a different storage number.

(5b) Answering Y will replace the method previously stored under that number with the Method presently shown on the screen display.

(5c) If the Method to be modified has been used in a chain, the prompt:

METHOD EXISTS IN CHAIN, OVERWRITE?

will appear.

To preserve the unmodified Method, type N.

Answering N exists from the STORE program. Repeat the store procedure assigning a different storage number.

Answering Y will overwrite the original Method.

4.11.5. Recalling A Stored Method To The Screen

(1) Recall the stored Method to the screen by pressing the RESET key and holding it down while pressing the METH key. The prompt will ask for the number of the Method to be recalled.

(2) Type in the number of the Method to be called to the screen from storage and press the ENTER key.

4.11.6. Running The Gradient Analysis Method

The Method to be run must be displayed on the Control Module screen. If another method is currently displayed, see the instructions for recalling a method from storage or generate the Method according to the instructions given above.

> Note: Always check the current values displayed on the bottom portion of the screen against the Method at the top of the screen before starting a new Method to make sure that an abrupt or improper solvent change which might damage the column will not be made by the Series 4.

(1) To start the pump, press the RESET key and hold it down while pressing the START key.

The pump will start and the system will equilibrate for the time entered in Step 3 above (EQUIL TIME). When the equilibration time has passed, the system will become "Ready" and the READY Light on the pump Module will light. The pump will continue to run, delivering the mixture defined in the EQUIL Sector, for the time entered in the READY TIME parameter unless the RUN button is pressed.

(2) Inject the sample.

(3) Immediately press the RUN button on the Pump Module.

Starting with Sector 1, the system will step through the sectors and then will return to the equilibration sector. When the equilibration times out, the READY light illuminates on the Pump Module.

If the RUN button on the Pump Module is pressed again, the program will step through the sectors, and automatically return to the equilibration sector. When equilibration times out again, the Series 4 becomes "Ready" again.

If the RUN button on the pump module is not pressed, the system will remain "Ready" and the pump will run for the time entered as READY TIME and then the Series 4 will shut down.

If the oven is connected to the Series 4 and is heated, the Series 4 will enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. The cool-down sequence protects the column. When it is complete, the Series 4 will shut down.

4.11.7. Stepping To The EQUIL Sector

A Gradient Method may be modified before the run is initiated, and while the system is in "EQUIL". The Method may not be modified while the Method is Ready or Running.

To step to the EQUIL Sector, press the RESET key and hold it down while pressing the blue EQUIL key. The System will step immediately to the EQUIL sector.

4.11.8. Modifying A Gradient Analysis Method

The Method to be modified must be displayed. If it is not presently displayed, call the Method to the screen before continuing with the instructions below.

To Modify the Gradient Method:

(1) Press the RESET key and hold it down while pressing the MOD key.

(2) Use the ENTER key to step through the parameters, making changes as necessary; or, press the RESET key and the appropriate parameter key (e.g., T1, T5, etc.) to step directly to the parameter to be changed.

When modifications are complete, the terminal will beep and the prompt will remind the user to store the Modified Method.

4.11.9. Using The Hold Key

The HOLD key may be used to hold the program in the current sector of a gradient analysis. The pump does not stop when this key is used but the internal clock does. The HOLD key, in effect, adds time to the current sector.

Press the RESET key and hold it down while pressing the HOLD key. To resume the programmed timing, press the HOLD key again.

4.11.10. Using The Pause Key

The PAUSE key may be used to temporarily pause the system. The PAUSE key stops the pump, as well as the internal clocks and timers. When the system is restarted, the program will resume where it paused.

To pause the system, press the RESET key and hold it down while pressing the PAUSE key. To restart the system press the PAUSE key again. The system will restart and the program will resume where it paused.

4.11.11. Stopping The Gradient Analysis

Press the RESET key and hold it down while pressing the STOP key. This will abort an analysis in progress.

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

4.11.12. Using A Gradient Method In A Chain

See Section 4.14. for instructions on Chaining Methods for unattended analysis.

4.12. FLOW PROGRAM ANALYSIS

4.12.1. Introduction To Flow Program Analysis

A flow program, or a gradual change in the flow rate of the mobile phase, is commonly used to reduce elution time of later peaks in a chromatogram. In some cases, a flow program can be used to reduce detector upset or mobile phase precipitation with an isocratic analysis. A flow program can eliminate the re-equilibration time and accelerated column wear associated with gradient operation in some analyses. A flow program can also be combined with a gradient program when you are limited in gradient capability because of buffer solubility, etc. but still need a shorter analysis time.

A concave flow program (curves 2, 3, 4 and 5) is used to maintain early resolution and sweep off later components. A convex flow program (curves 0.2, 0.3, 0.4 and 0.5) is used to speed up the early peaks and maintain resolution of the later peaks. The curves for a flow program are shown in Figure 4-16. As with solvent gradients, the most useful flow programs are the linear and concave curves.

Once a method has been developed and the optimum parameters for a satisfactory analysis are known, the user can store the flow program Method for later use and for inclusion in a Method Chain for unattended analysis.

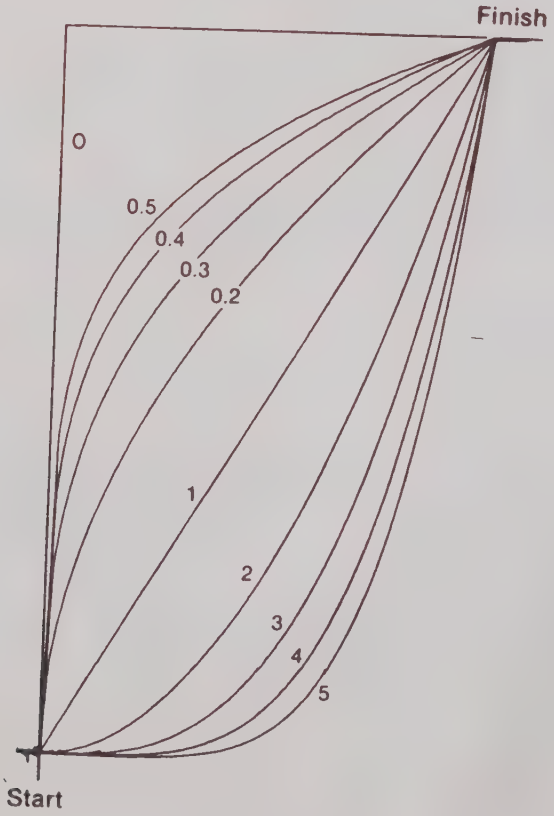


Figure 4-16 - Flow Curves Available on the Series 4

4.12.2. Modifying An Existing Method

It is possible to modify an existing Method to create a Flow Program Method, but normally a new Flow Program Method would be generated.

The Method to be modified must be displayed on the Control Module screen.

Press the RESET key and hold it down while pressing the MOD key.

The Series 4 enters the MODIFY mode. The prompt

SELECT SECTOR OR PRESS ENTER KEY

willl appear.

Press the appropirate parameter key or press the ENTER key to step through the parameters.

See below for instructions on Flow Program Parameter Entries.

When all parameter entries have been made, the Series 4 will leave the MODIFY Mode automatically. The terminal will beep and the prompt will remind the user to store the Method.

4.12.3. Generating A Flow Program Method

(1) Press the RESET key and hold it down while pressing the GEN key. The prompt line will say:

PRESS METHOD, CHAIN, OR CE

(2) Press the METH key. The prompt:

CLEAR CURRENT METHOD (Y/N)?

will appear.

(3) Type Y. The Screen will clear and the Method Generation Screen Display will appear. the prompt line on the Screen Display will read:

ENTER METHOD DESCRIPTION

You are now ready to enter the Flow Program Method parameters. Enter or default the parameters as desired (see Flow Program Parameter Entries, below).

When all parameter entries have been made, the Series 4 will leave the Method Generation mode automatically. The terminal will beep and the prompt will remind the user to store the Method.

Figure 4-17 shows the Method Screen Display for an Isocratic Flow Program Analysis. The display is divided into 3 sections: the Current Method Section, the Current Status Section and the line for prompts, queries and error messages.

```
METHOD    ISOCRATIC FLOW PROGRAM                              TIME 20.0 MINS

SECTOR    TIME    FLOW        ACN       H2O
                  MLS CURVE   A%        B%        C%        D%        CURVE

EQUIL      5.0    2.0         80.0      20.0
  1       10.0    4.0    1    80.0      20.0
  2        5.0    4.0         80.0      20.0
  3        0.0
  4
  5
READY TIME       60.0 MIN     0.5   MPa  PMAX   42.0 MPa  TEMPERATURE  40


VIAL    INJECTION          TOTAL              CURRENT              PRESSURE
          NO.              TIME          SECTOR       TIME          MPa
                           0.4           EQUIL        0.4           21.2

                  FLOW        ACN       H2O

CURRENT VALUES    2.0         80.0      20.0
```

Figure 4-17 - A Sample Screen Display for an Isocratic Flow Program

```
METHOD   GRADIENT FLOW PROGRAM                                   TIME  3.4 MINS

SECTOR    TIME    FLOW        ACN       MEOH      PO4       B-6
                  MLS CURVE   A%        B%        C%        D%        CURVE

EQUIL      1.3    2.0         38.0      10.0       3.0      49.0
  1        2.1    3.0    1    58.0      10.0       3.0      29.0         1
  2        0.0
  3
  4
  5
READY TIME       60.0  PMIN   1.8  PMAX  24.0 MPa          TEMPERATURE 40


VIAL    INJECTION          TOTAL              CURRENT              PRESSURE
          NO.              TIME          SECTOR       TIME          MPa
                           1.4           READY        0.0           21.8

                  FLOW        ACN       MEOH      PO4       B-6

CURRENT VALUES    2.0         38.0      10.0       3.0      49.0
```

Figure 4-18 - Sample Screen Display Showing a Combined Gradient and Flow Program Method

The Current Method is always shown at the top of the screen unless it is temporarily replaced by the Chain display or the Directory because of an operator request.

The Current Status section is displayed after Method Generation or Modification is complete. It is continuously and automatically updated so that the operator can see at a glance what is happening.

The last line is used for prompts and messages to the user.

4.12.4. Parameter Entry For A Flow Program

1. METHOD DESCRIPTION

   Type in a description of the Method and press the ENTER key or merely press the ENTER key to default the entry and step to the next parameter. Up to 40 alpha-numeric characters may be used.

2. SOLVENT RESERVOIRS

   The cursor will step to the first reservoir identification position (Reservoir A). Enter an identification for this reservoir (e.g., H20, ACN, MEOH, THF) if it is to be used. Up to 5 alpha-numeric characters may be entered. Default the entry by pressing the ENTER key if this reservoir is not to be used. A reservoir must be identified in order to use it in the Method. Each of the 4 reservoirs should either be identified or defaulted.

   For a two-solvent mixture, you would identify and use two reservoirs, A and B, for example. For a four-solvent mixture, all four reservoirs must be identified.

EQUILIBRATION SECTOR

3. EQUIL TIME

   Type in an equilibration time and press the ENTER key or default this entry by pressing the ENTER key. The default value is 10 min. If a solvent change is made, the equilibration time should be long enough to allow the column to equilibrate with the new solvent (at least 10 column volumes should be used) and for the temperature to equilibrate if the column oven is to be used. Permissible values are 0.1 to 999.9 minutes. The system will not become "Ready" until the equilibration time entered has passed.

   Note: If the Method is used in a chain where a solvent change necessitates a longer equilibration time, the longer equilibration time should be added to the method which follows the solvent change.

4. FLOW RATE (MLS)

   Type in a flow rate for the equilibration sector in ml/min. and press the ENTER key or just press the ENTER key to obtain the default flow rate of 2 ml/min. This entry may be any number between 0.1 and 9.9.

5. % SOLVENT

The cursor will step to the data entry position for entering the percentage of solvent to be taken from the first reservoir identified in step 2, above. Type in the percent solvent to be used from that reservoir and press the ENTER key. Enter the percent solvent for each identified reservoir except the last one--the system will automatically calculate the percentage from the last identified reservoir so that the sum of the percentages will equal 100%.

For example, if only two reservoirs were identified in step 2, when 60% is entered for the first reservoir, the system will automatically enter 40% for the second reservoir. If four reservoirs were identified in step 2 but only two are to be used in the method, the percentages for both must be entered by the user. If the solvent is to be used from 4 identified reservoirs, upon entry of the third percentage, the system will calculate the fourth percentage.

If the user attempts to enter percentages totalling more than 100%, the Series 4 will beep and the prompt:

ERROR, CE
or
TOTAL PERCENT EXCEEDS 100%

will appear. Re-enter the proper percentages.

SECTOR ENTRIES

The Series 4 has five different independently programmable time sectors. For each of these sectors, the user can specifiy time, flow rate and flow curve, percent composition for each solvent reservoir and the gradient curve if a gradient is to be used. Two separate curves are allowed in each sector: one flow rate change and one percent composition change. (See Operator Aids Section - How the Series 4 Counts Curves.)

After the EQUIL sector entries are completed, the cursor will go to the Sector 1 TIME entry position then to FLOW RATE (MLS) and FLOW CURVE and through % SOLVENT for each identified reservoir, to curve. Then Sector 2 entries are made, then Sector 3 entries, and so forth. Pressing the ENTER key when the cursor is in the Sector TIME entry position enters 0, which signifies to the Series 4 that no other sectors are to be used. Steps 6 through 10 describe sector parameter entries.

SECTOR 1-5 ENTRIES

6. TIME 1

Type in the time over which the change in the mobile phase in this sector of the Method is to occur. If you refer to Figure 4-17, you will see that 5 minutes has been entered for equilibration time, and that 10 minutes has been entered under TIME in Sector 1. The 10 minutes entered in Sector 1 means that the change in flow rate from 2 ml/min. to 4 ml/min. will take 10 minutes to complete. Permissible values are 0.1 to 999.9 minutes.

7. FLOW RATE (MLS)

Type in a flow rate in ml/min. for the sector of the Method and press the ENTER key or press the ENTER key to obtain the default flow rate of 2 ml/min. This entry may be any value between 0.1 and 9.9.

8. FLOW RATE (CURVE)

If the flow rate entered in Sector 1 differs from that used in the EQUIL sector, or if the flow rate is changed in subsequent Sectors, the cursor will step to the FLOW CURVE data entry position. Type in the number for the flow curve and press the ENTER key. A linear flow curve is 1. Convex flow curves are 0.2, 0.3, 0.4 and 0.5. Concave flow curves are 2, 3, 4, and 5. These curves are shown in Fig. 4-16.

9. % SOLVENT

The cursor will step to the entry position for the first reservoir identified in step 2, above. Type in the percent solvent from that reservoir to be used and press the ENTER key. The cursor will step to the entry position for the next reservoir identified in step 2, above. Enter the percent solvent to be used and press the ENTER key. Repeat this for each identified reservoir.

10. CURVE

If a solvent gradient is used, type in the number for the desired gradient curve and press the ENTER key. A linear gradient curve is 1. Convex gradients are 0.2, 0.3, 0.4 and 0.5. Concave gradients are 2, 3, 4 and 5. A maximum of 2 curves is permissible in any time sector. (See the Operator Aids Section - How the Series 4 Counts Curves.)

The Series 4 will now step to the next sector. Repeat the entries (6-10) for each sector to be used. After all sector entries have been made, enter 0 under time in the next sector. This indicates the end of the sector entries to the Series 4.

11. READY TIME

Type in a READY TIME and press the ENTER key or default the entry by pressing the ENTER key. Permissible values are 0.1 to 999.9 The default value is 60 min. Ready time is the amount of time the system will remain "ready" and waiting to run without shutting down.

> **Note:** A Ready Time of zero (0) minutes or infinite time must NOT be used since entering zero will automatically change the program to the Untimed Isocratic mode and erase all entries made in sectors 1-5.

12. PMIN

Type in a value for the minimum pressure and press the ENTER key or default the entry by pressing the ENTER key. The default value for PMIN is zero (0). Typical values are 0 to 2 MPa. Permissible values are 0.0 to 41.9 MPa. The PMIN entered must be less than the PMAX entry (see below).

> **Note:** If the system does not reach PMIN +10% within one minute, the Series 4 will assume that a leak exists or that the solvent reservoir is empty, and the pump will automatically shut down. It is therefore advisable to enter a value for PMIN greater than zero when the System is to run unattended.

13. PMAX

Type in a value for the maximum pressure which will protect the column(s) used in the analysis from excessive pressure or default the entry by pressing the ENTER key. If the value for PMAX is reached, the system will automatically shut down.

Any value between 0.1 and 42 MPa may be entered for PMAX. The default value for this entry is 42 MPA (6000 psi). This is normally used unless the column is fragile.

If a value is entered for PMAX which is less than or equal to the value entered for PMIN, the Control Module will beep, and the prompt will ask for a new value for PMAX.

14. TEMPERATURE

If the optional column oven is connected, type in a value between 35°C and 99°C and press the ENTER key or default the parameter by pressing the ENTER key. The default value is OFF. If the value entered is below 35°C, the system will automatically default to power OFF.

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

CAUTION: The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

The parameter entries are now complete. The terminal will beep and the prompt will remind the user to store the Method.

4.12.5. Storing A Flow Program Analysis

(1) Check the screen display to see that the required parameters have been entered correctly.

(2) Press the RESET key and hold it down while pressing the STORE key.

(3) The screen prompt will request:

ENTER METHOD STORAGE NUMBER

(4a) The user may enter a number from 1 to 9 or press the ENTER key to cause the system to assign the next available number to the method to be stored.

(4b) Entering a number which has already been used will cause the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

to appear.

(5a) Answering N will exit from the STORE program. Repeat the Store procedure, assigning a different storage number.

(5b) Answering Y will replace the method stored under that number with the method presently shown on the screen display.

(5c) If the Method to be modified has been used in a chain, the prompt:

METHOD EXISTS IN CHAIN, OVERWRITE (Y/N)?

will apear.

To preserve the unmodified Method, type N.

Answering N exits from the STORE Program. Repeat the STORE Procedure, assigning a different storage number.

Answering Y will overwrite the original Method.

4.12.6. Recalling A Stored Method To The Screen

(1) Recall the stored Method to the screen by pressing the RESET key and holding it down while pressing the METH key. The prompt will ask for the Method number.

(2) Type in the number of the Method to be called to the screen from storage and press the ENTER key.

4.12.7. Running The Flow Program

The Method to be run must be displayed on the Control Module screen. If another method is currently displayed, see the instructions for recalling a method from storage or generate the Method according to the instructions given above.

(1) To start the pump, press the RESET key and hold it down while pressing the START key.

The pump will start and the system will equilibrate for the time entered as EQUIL TIME. When the equilibration time has passed, the system will become "Ready" and the READY light on the Pump Module will light. The pump will continue to run, delivering the mixture defined in EQUIL sector for the time entered in the READY TIME parameter unless the RUN button is pressed.

(2) Inject the sample.

(3) Immediately press the RUN button on the Pump Module.

Starting with sector 1, the system will step through the sectors and then will return to the equilibration sector. When the equilibration times out, the READY light illuminates on the Pump Module.

If the RUN button on the Pump Module is pressed again, the program will step through the sectors, automatically return to the equilibration sector, and, when equilibration times out, the Series 4 becomes "Ready" again.

If the RUN button on the pump module is not pressed, the system will remain "Ready" and the pump will run for the time entered under READY TIME and then the Series 4 will shut down.

If the oven is connected to the Series 4 and is heated, the Series 4 will enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. This cool-down sequence protects the column. when it is complete, the Series 4 will shut down.

4.12.8. Stepping To The EQUIL Sector

A Flow Program method may be modified before the run is initiated, and while the system in in "EQUIL".

If the System is not in shut down or EQUIL, press the RESET key and hold it down while pressing the blue EQUIL key. The program will immediately step to the EQUIL sector.

4.12.9. Modifying A Flow Program Method

The Method to be modified must be displayed on the Control Module. If it is not presently displayed, call the Method to the screen before continuing the instructions below.

(1) Press the RESET key and hold it down while pressing the MOD key.

(2) Use the ENTER key to step through the parameters, making changes as necessary; or, press the RESET key and the appropriate parameter key (e.g., T1, T5, etc.) to step directly to the parameter to be changed.

When modification is complete, the terminal will beep and the prompt will remind the user to store the Modifications.

4.12.10. Using The Hold Key

The HOLD key may be used to hold the program in the current sector of a Gradient or Flow Program analysis. The internal clock stops, but the pump continues to run when this key is used. The HOLD key, in effect, adds time to the current sector.

To use the HOLD key, press the RESET key and hold it down while pressing the HOLD key. To resume the programmed timing, press the RESET key and hold it down while pressing the HOLD key again.

4.12.11. Using The Pause Key

The PAUSE key may be used to temporarily pause the system. All the internal clocks and timers on the Series 4 will pause and the pump will stop. When the system is restarted, the program will resume where it paused.

To pause the system, press the RESET key and hold it down while pressing the PAUSE key. To restart the system, press the RESET key and hold it down while pressing the PAUSE key again. The system will restart and the program will resume where it paused.

4.12.12. Stopping The Flow Program Analysis

Press the RESET key and hold it down while pressing the STOP key. This will abort an analysis in progress.

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. when the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. do not accidentally use the STOP key to stop the pump duirng the cool-down sequence.

4.12.13. Using A Flow Program Method In A Chain

See Section 4.14. for instructions on Chaining Methods for unattended analysis.

4.13. COMBINING A GRADIENT PROGRAM WITH A FLOW PROGRAM

It is sometimes advantageous to combine a gradient program with a flow program when you are limited in gradient capability because of buffer solubility, etc. but still need a shorter analysis time.

The instructions for modifying or generating a program for a combined Gradient and Flow Program are the same as those given in Sections 4.11 and 4.12 except for parameter entries.

4.13.1. Parameter Entry For A Combined Gradient Program And Flow Program

1. METHOD DESCRIPTION

   Type in a description of the Method and press the ENTER key or merely press the ENTER key to default the entry and step to the next parameter. Up to 40 alpha-numeric characters may be used.

2. SOLVENT RESERVOIRS

   The cursor will step to the first reservoir identification position (Reservoir A). Enter an identification for this reservoir (e.g., H2O, ACN, MEOH, THF) if it is to be used. Up to 5 alpha-numeric characters may be entered. Default the entry by pressing the ENTER key if this reservoir is not to be used. A reservoir must be identified in order to use it in the Method. Each of the 4 reservoirs should either be identified or defaulted.

   For a two-solvent mixture, you would identify and use two reservoirs, A and B, for example. For a four-solvent mixture, all four reservoirs must be identified.

EQUILIBRATION SECTOR

3. EQUIL TIME

Type in an equilibration time and press the ENTER key or default this entry by pressing the ENTER key. The default value is 10 min. If a solvent change is made, the equilibration time should be long enough to allow the column to equilibrate with the new solvent (at least 10 column volumes should be used) and for the temperature to equilibrate if the column oven is to be used. Permissible values are 0.1 to 999.9 minutes. The system will not become "Ready" until the equilibration time entered has passed.

Note: If the Method is used in a chain where a solvent change necessitates a longer equilibration time, the longer equilibration time should be added to the method which follows the solvent change.

4. FLOW RATE (MLS)

Type in a flow rate for the equilibration sector in ml/min. and press the ENTER key or just press the ENTER key to obtain the default flow rate of 2 ml/min. This entry may be any number between 0.1 and 9.9.

5. % SOLVENT

The cursor will step to the data entry position for entering the percentage of solvent to be taken from the first reservoir identified in step 2, above. Type in the percent solvent to be used from that reservoir and press the ENTER key. Enter the percent solvent for each identified reservoir except the last one--the system will automatically calculate the percentage from the last identified reservoir so that the sum of the percentages will equal 100%.

For example, if only two reservoirs were identified in step 2, when 60% is entered for the first reservoir, the system will automatically enter 40% for the second reservoir. If four reservoirs were identified in step 2 but only two are to be used in the method, the percentages for both must be entered by the user. If the solvent is to be used from 4 identified reservoirs, upon entry of the third percentage, the system will calculate the fourth percentage.

If the user attempts to enter percentages totaling more than 100%, the Series 4 will beep and the prompt:

ERROR, CE
or
TOTAL PERCENT EXCEEDS 100%

will appear. Re-enter the proper percentages.

SECTOR ENTRIES

The Series 4 has five different independently-programmable time sectors. For each of these sectors, the user can specify time, flow rate and flow curve, percent compostion for each solvent reservoir and the gradient curve if a gradient is to be used. Two separate curves are allowed in each sector: one flow rate change and one percent composition change. (See the Operator Aids Section - How the Series 4 Counts Curves.)

SECTOR 1-5 ENTRIES

6. TIME

Type in the time over which the change in the mobile phase in this sector of the Method is to occur. Permissible values are 0.1 to 999.9 minutes.

7. FLOW RATE (MLS)

Type in a flow rate in ml/min. for the sector of the method and press the ENTER key or press the ENTER key to obtain the defualt flow rate of 2 ml/min. This entry may be any value between 0.1 and 9.9.

8. FLOW RATE (CURVE)

Whenever the flow rate entered in step 7 differs from the flow rate entered in the previous sector, the cursor will step to the FLOW CURVE data entry position. Type in the number for the flow curve and press the ENTER key. A linear flow curve is 1. Convex flow curves are 0.2, 0,3, 0.4 and 0.5. Concave curves are 2, 3,4 and 5. These curves are shown in Figure 4-16.

9. % SOLVENT

The cursor will step to the entry position for the first reservoir identified in step 2, above. Type in the percent solvent from that reservoir to be used and press the ENTER key. the cursor will step to the entry position for the next reservoir identified in step 2, above. Enter the percent solvent to be used and press the ENTER key. Repeat this for each identified reservoir.

10. CURVE

If a solvent gradient is used, type in the number for the desired gradient curve and press the ENTER key. A linear gradient curve is 1. Convex gradients are 0.2, 0.3, 0.4 and 0.5. Concave gradients are 2, 3, 4 and 5. A maximum of 2 curves is permissible in any time sector. (See the Operator Aids Section -How the Series 4 Counts Curves.)

The Series 4 will now step to the next sector. Repeat the entries (6-10) for each sector to be used. After all sector entries have been made, enter 0 under time in the next sector. This indicates the end of the Sector entries to the Series 4.

11. READY TIME

Type in a READY TIME and press the ENTER key or default the entry by pressing the ENTER key. Permissible values are 0.1 to 999.9. The default value is 60 min. Ready time is the amount of time the system will remain "ready" and waiting to run without shutting down.

> **Note:** A Ready Time of zero (0) minutes or infinite time must NOT be used since entering zero will automatically change the program to the untimed isocratic mode and erase all entries made in sectors 1-5.

12. PMIN

Type in a value for the minimum pressure and press the ENTER key or default the entry by pressing the ENTER key. The default value for PMIN is zero (0). Typical values are 0 to 2 MPa. Permissible values are 0.0 to 41.9 MPa. the PMIN entered must be less than the PMAX entry (see below).

> **Note:** If the system does not reach PMIN within one minute, the Series 4 will assume that a leak exists or that the solvent reservoir is empty, and the pump will automatically shut down. It is therefore advisable to enter a value for PMIN greater than zero when the System is to run unattended.

13. PMAX

Type in a value for the maximum pressure which will protect the column(s) used in the analysis from excessive pressure or default the entry by pressing the ENTER key. If the value for PMAX is reached, the system will automatically shut down.

Any value between 0.1 and 42 MPa may be entered for PMAX. The default value for this entry is 42 MPa (6000 psi). This is normally used unless the column is fragile.

If a value is entered for PMAX which is less then or equal to the value entered for PMIN, the LC Terminal will beep, and the prompt will ask for a new value for PMAX.

14. TEMPERATURE

If the optional column oven is connected, type in a value between 35^{o}C and 99^{o}C and press the ENTER key or default the parameter by pressing the ENTER key. The default value is OFF. If the value entered is below 35^{o}C, the system will automatically default a power OFF condition.

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automaticlaly enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. when the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

The parameter entries are now complete.

4.13.2. Two Curves Per Sector

Two curves are allowed in each time sector on the Series 4. Thus, only one flow rate change and one solvent gradient will be allowed in any one sector.

Since the last identified reservoir is always used to make up the residual to insure that the total percentages always equal 100%, a change in solvent composition in this last reservoir is not counted as a curve. Therefore, if only one solvent curve is to be run in a sector, the last identified reservoir must be used to balance the curve.

See the Operator Aids Section of this manual for a discussion of How The Series 4 Counts Curves.

4.14. CHAINING METHODS TOGETHER FOR UNATTENDED ANALYSES

If you have an autosampler, up to 9 user-stored methods may be chained together in any order and for any number of times to analyze up to 100 individual samples with up to 9 injections of each sample.

To connect the Autosampler to the Series 4, see the Installation Section.

4.14.1. Generating A Chain

A chain is generated after all the methods to be used in the Chain have been stored in the Series 4 memory.

(1) Press the RESET key and hold it down while pressing the GEN key. The prompt will say:

PRESS METHOD, CHAIN, OR CE

(2a) Press the CHAIN key. If no Chain exists, the prompt will say:

CLEAR CURRENT METHOD (Y/N)?

(2b) If a Chain already exists, the prompt

CHAIN EXISTS, OVERWRITE (Y/N)?

will appear.

(3a) Type Y unless the Current Method has not yet been stored and you want to save it. Typing N will exit from the Chain Generation routine and allow you to store the current Method.

(3b) Type Y to Overwrite the existing Chain.

Typing N will exit from the Chain Generation mode.

The screen will clear and the CHAIN Screen Page (see Figure 4-19) will appear.

```
NO. OF        VIAL      NO. OF   METHOD     METHOD DESCRIPTION
SAMPLES       NO.       INJECT
              F    L

GEN>PRESS RESET/STORE TO EXIT CHAIN GENERATION ■
```

Figure 4-19 - CHAIN Generation Screen Page

(4) The first entry required is the number of samples to be analyzed with the first Method in the Chain.

Type the number of samples to be analyzed with the first Method and press the ENTER key.

The system will automatically enter the first and last vial numbers in the second and third columns of the Chain Screen Display. The Autosampler should be loaded according to this display.

(5) The second entry required is the number of injections to be made of each sample.

Type the number of injections and press the ENTER key.

(6) The third entry required is the number of the Method to be used to analyze the first group of samples.

Type in the Method number and press the ENTER key.

The system will automatically enter the Method Description and the system will step to the next line.

> Note: The Method number to be entered is the number the Method is stored under in Series 4 memory. If a Method number is entered which has not been used to store a Method in memory the following prompt will appear:
>
> METHOD DOES NOT EXIST, CE
>
> Press the CE key and then re-enter the correct Method number.
>
> **Note:** The Directory can be called at any time to remind the user of Methods stored and their numbers.

(7) The first entry on the second line is the number of samples to be analyzed with the second Method in the Chain.

Type the number of samples to be analyzed with the second Method and press the ENTER key.

The system will automatically enter the first and last vial numbers to be used in the second and third columns of the Chain Screen Display.

(8) The second entry on the second line is the number of injections to be made of each sample to be analyzed with the second Method.

Type in the number of injections and press the ENTER key.

(9) The third entry on the second line is the number of the Method to be used to analyze the second group of samples.

Type in the Method number and press the ENTER key.

The system will automatically enter the Method Description and the system will step to the next line.

This entry procedure is continued until all the samples, the number of injections of each and the Method to be used to analyze them have been specified.

CHAINING METHODS WHICH USE THE OVEN

When chaining together several methods which use the OVEN, the Methods should be arranged in the Chain so that the temperature increases as the Chain progresses. All Methods which do not use the OVEN (temperatures below 35°C) should come before Methods which use temperature control.

When a timed run is programmed with a heated oven, when the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

4.14.2. Storing The Chain

When the Chain has been generated, the Screen Display will be similar to the example shown in Figure 4-20. To end the Chain Generation, press the RESET key and hold it down while pressing the STORE key.

When the Chain has been stored, the prompt

PRESS RESET/METHOD OR RESET/DIR

will appear.

```
NO. OF        VIAL        NO. OF    METHOD     METHOD DESCRIPTION
SAMPLES       NO.         INJECT
              F    L

   6          1    6        1         2     TEST 3
   4          7   10        1         1     TEST 2


GEN>PRESS RESET/STORE TO EXIT CHAIN GENERATION ■
```

Figure 4-20 - Sample Chain Screen Display after entries have been made and Chain has been stored.

4.14.3. Running A Chain

4.14.3.1. STARTING THE METHOD

To run the Chain, the first Method in the Chain must be displayed on the screen and must be running in the EQUIL or READY time Sectors.

(1) Press the RESET key and hold it down while pressing the METH key.

The prompt will say:

ENTER METHOD NUMBER

(2) Type in the Method storage number of the first Method in the Chain and press the ENTER key. The screen will clear and the Method Screen Display for the specified Method will appear.

(3) Press the RESET key and hold it down while pressing the START key. The pump will start and the system will go to the EQUIL sector.

4.14.3.2. EXECUTING THE CHAIN

Press the RESET key and hold it down while pressing the EXEC key. This will begin the Chain process.

Note: EXEC does not put the first Method into RUN: it just implements the Chain. The user must press the RUN Button to begin the first run. The Autosampler will give the RUN signal for all succeeding analyses.

Notice that in the Current Status section of the Screen Display the Vial Number and Injection Numbers will be filled in by the system and "Chain Running" will appear next to CURRENT SECTOR.

> **Note:** Unless the Series 4 is connected so that it receives an external RUN signal (see the Installation Section), it will be necessary to press the RUN button each time the system becomes "Ready".

4.14.4. Stopping A Chain Run

4.14.4.1 EXITING FROM THE CHAIN

To exit from a CHAIN, press the RESET key and hold it down while pressing the EXIT key. This exits from the Chain, but will not stop any analysis in progress.

> **Important:** It is not possible to exit from a Chain and re-enter it subsequently at the exit point. Either the entire Chain must be rerun from the beginning or the Chain must be modified so that the first Method to be run is the first Method in the Chain.

4.14.4.2 STOPPING THE METHOD

To stop an analysis in progress, press the RESET key and hold it down while pressing the STOP key.

When a timed run is programmed with a heated oven, when the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. when the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

4.14.5. Recalling A Chain Program

Recall the stored Chain to the screen by pressing the RESET key and holding it down while pressing the CHAIN key.

4.14.6. Modifying A Chain

A Chain may not be modified while it is running.

(1) Press the RESET key and hold it down while pressing the CHAIN key.

(2) When the Chain is displayed, press the RESET key and hold it down while pressing the MOD key. The prompt will read:

ENTER FIRST VIAL NUMBER OF LINE TO BE MODIFIED

(3) Type in the first vial number in the line you wish to modify. The cursor will step to the line to be changed. Use the ENTER key to step to the data entry position and make all necessary changes.

The modifications will be stored automatically.

To make another modification, repeat steps (2) and (3) above.

To exit from the Chain Modification Program, follow the directions of the prompt:

PRESS RESET/METH OR RESET/DIR

> **Important:** It is not possible to exit from a Chain and re-enter it subsequently at the exit point. Either the entire Chain must be re-run from the beginning or the Chain must be modified so that the first Method to be run is the first Method in the Chain. If a Chain must be temporarily modified, it is advisable to use the blank screen pages (see the Operator Aids Section of this manual) to record the complete Chain.

4.15. OPERATING WITHOUT SOLVENT PRESSURIZATION

For short-term operation (less than 2 hours) solvents may be vacuum or ultrasonic degassed using conventional technques and used immediately before they dissolve gas from the environment.

To operate in this manner with the Series 4, the door to the pressurization chamber must be left open so that the reservoirs are not sealed by the door-operated interlock valve.

4.16. OPERATING WITH DUPLICATE SOLVENTS FOR LONGER OPERATION

To extend the time the Series 4 will operate without refilling the reservoirs, if a two-solvent analysis is to be performed, all four solvent reservoirs may be filled. For example, if the Method calls for a 60/40 ratio of the two solvents, 2 solvent reservoirs could be filled with each of the 2 solvents to be used, and in the Method, all four reservoirs would be identified, with half of each solvent being taken from each reservoir, as in the following example:

```
METHOD   LONGER RUNNING TIME 60/40                          TIME 10.0 MINS

SECTOR    TIME    FLOW        MEOH      MEOH      H2O       H2O
                  MLS CURVE   A%        B%        C%        D%        CURVE

EQUIL     10.0    2.0         30.0      30.0      20.0      20.0
  1        0.0
  2
  3
  4
  5
READY TIME      INF    PMIN   0.0   MPa  PMAX   42.0 MPa  TEMPERATURE OFF
```

Figure 4-21a - Operating with Duplicate Solvents - 2 Reservoirs Each

If the method calls for a higher ratio of one solvent to the other, it is possible to fill 3 reservoirs with one of the solvents and other reservoir with the second solvent as in the example below.

```
METHOD   LONGER RUNNING TIME 90/10                          TIME 10.0 MINS

SECTOR    TIME    FLOW          ACN        ACN        ACN        H2O
                  MLS CURVE     A%         B%         C%         D%        CURVE

EQUIL     10.0    2.0           30.0       30.0       30.0       10.0
  1        0.0
  2
  3
  4
  5
READY TIME      INF    PMIN   0.0   MPa  PMAX  42.0  MPa  TEMPERATURE OFF
```

Figure 4-21b - Using 3 Reservoirs for one of the Solvents

4.17. SOLVENT RESERVOIR CHANGEOVER

When making solvent reservoir changes, it is advisable to clean out the filter and the sparger to avoid contaminating the new solvents.

4.17.1. Cleaning the Inlet Filter and Sparger

(1) Unscrew the caps from the solvent reservoirs by turning the reservoirs while holding the caps to avoid twisting the lines.

(2) Remove the solvent reservoirs from the pressurization chamber.

(3) Place the solvent input lines and the sparger lines in beakers.

(4) Turn on the helium and blow through the lines to clear the spargers.

(5) Turn off the helium.

(6) Remove the sparger and solvent lines from the beaker. Run the Series 4 through a Purge cycle for each solvent input line which is to be cleared.

Pull air through each line which is to be cleared with the syringe.

(7) Place each solvent input and sparger line pair in a 250 ml beaker containing 100 ml of the new solvent which will be used in that reservoir.

(8) Purge the system with the new solvent(s), allowing approximately 30-60 ml of each new solvent to flow through the complete system.

(9) Place the sparger and filter pair into the reservoir containing the new solvent and tighten the caps.

(10) Purge each solvent line again before restarting the system.

4.17.2. Solvent Miscibility

Make sure that the old solvent and the new solvent are miscible. If they are not miscible, an intermediate solvent which is miscible in both the old and the new solvent must be used for an intermediate purge. Isopropyl alcohol is most frequently used when drastic changes, such as from hexane to water, are made. For information on solvent miscibility, see the Solvent Polarity and Property Scale given in Appendix A.

5 CONTROLS, INDICATORS AND KEYBOARD

Section		Page
5.1	Series 4 Front Panel Controls	5-1
5.1.1	POWER ON/OFF Switch	5-1
5.1.2	READY/RUN Illuminated Pushbutton	5-1
5.2	Series 4 Internal Controls	5-3
5.2.1	Drain Valve Handle	5-3
5.2.2	Vent (Flush Port)	5-3
5.3	Series 4 Pressurization Controls	5-3
5.3.1	Pressure Regulator Valve	5-3
5.3.2	Pressure Gauge	5-3
5.3.3	Solvent Chamber Door Latch Knob	5-3
5.4	Series 4 Interface Control Panel	5-3
5.4.1	MANUAL RUN/AUTO Run Switch	5-3
5.4.2	Memory Protect Switch	5-4
5.4.3	Input and Output Contacts	5-4
5.5	Power Selection & System Fuse	5-5
5.5.1	Voltage Selection & System Fuse	5-5
5.5.2	Power Cord	5-6
5.5.3	Power Supply Fuse	5-6
5.6	LC Terminal - Front Panel Controls	5-7
5.6.1	ON/OFF Switch	5-7
5.6.2	Brightness Control	5-7
5.6.3	Line Key	5-7
5.6.4	Data Line Set-Up	5-8
5.6.5	Setting the Parity and the Baud Rate on the Series 4 Pump Module	5-9
5.7	LC Terminal - Rear Panel Controls	5-9
5.7.1	RS232 Connector Port	5-9
5.7.2	Printer Port	5-9
5.7.3	Power Cord	5-9
5.7.4	Fuse	5-9
5.8	Control Module Keyboard	5-10
5.8.1	General Purpose Keys	5-10
5.8.2	Mode Command Keys (Blue Keys, Upper Row)	5-12
5.8.3	Operational Command Keys (Blue Keys, Middle Row)	5-13
5.8.4	Chain Command Keys (Blue Keys, Lower Row)	5-14
5.8.5	Function Parameter Keys (White Keys)	5-15
5.9	Software-Controlled Password	5-16
5.10	Screen Layout	5-17

5 CONTROLS, INDICATORS AND KEYBOARD

5.1. SERIES 4 FRONT PANEL CONTROLS

5.1.1. POWER ON/OFF Switch

When the system is on, this switch is illuminated.

5.1.2. READY/RUN Illuminated Pushbutton

The Ready Light will illuminate when the system is ready; that is, when the user-entered Equilibration time has expired. When pressed, the Ready Light will extinguish, the Run Light will illuminate and the program will start at time segment 1 in the Method.

For use with totally automated systems the READY/RUN pushbutton may be inhibited. The external run input is used instead to initiate each run in a series of analyses.

If a run is started by an external device, the action will be the same as if the run were started with the READY/RUN pushbutton and the pushbutton will be illuminated in the same manner.

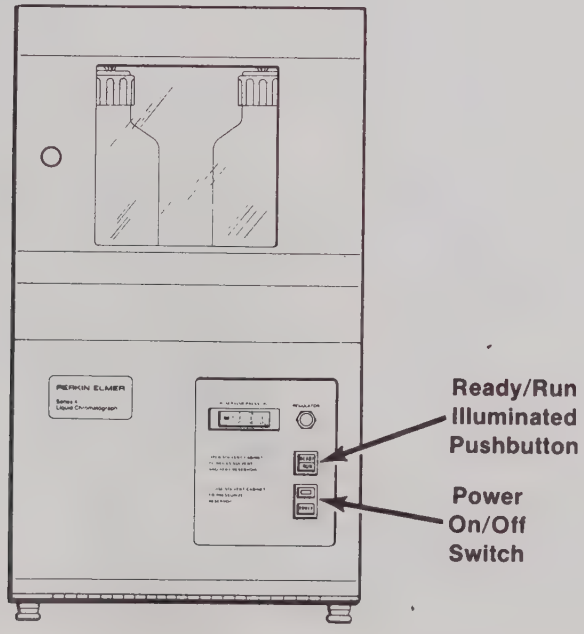


Figure 5-1 - Series 4 Front Panel Controls

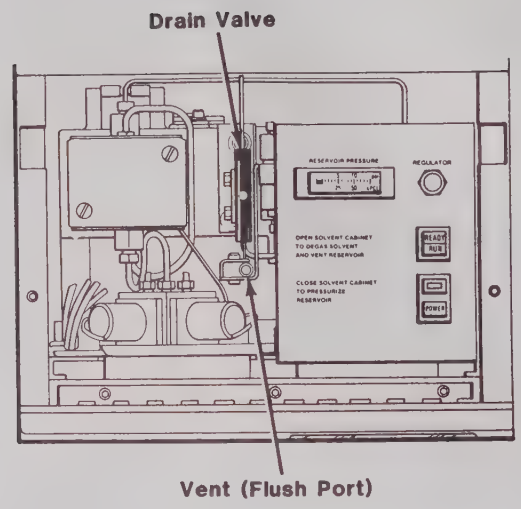


Figure 5-2 - Series 4 Internal Controls Showing Drain Valve and Vent Locations

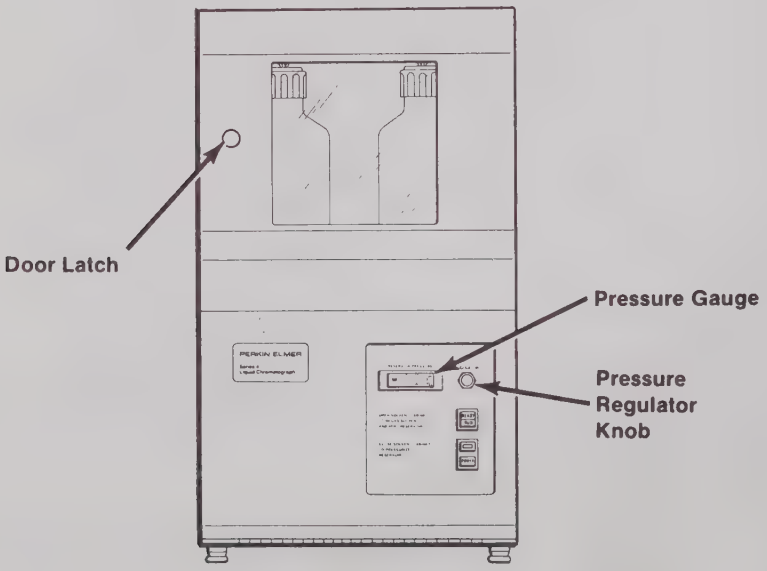


Figure 5-3 - Series 4 Pressurization Controls

5.2. SERIES 4 INTERNAL CONTROLS

5.2.1. Drain valve handle

Turning the handle counterclockwise permits solvent to travel from the pump to the vent (flush port) for the purpose of priming or purging.

5.2.2. Vent (Flush Port)

This outlet is provided for flushing and priming the pump. The vent fitting is threaded to accept the priming syringe supplied.

5.3. SERIES 4 PRESSURIZATION CONTROLS

5.3.1. Pressure Regulator Valve

Turning this knob counterclockwise closes the valve. Turning this knob clockwise increases the pressure applied to the reservoirs.

5.3.2. Pressure Gauge

This gauge indicates the helium pressure inside the Solvent Pressurization Chamber.

5.3.3. Solvent Chamber Door Latch Knob

When the door to the Solvent Pressurization Chamber is open, the solvent reservoirs are depressurized . When the door is closed, the solvent reservoirs will pressurize.

> **Warning:** Whenever the door to the solvent chamber is opened, the solvent reservoirs depressurize automatically. This is a safety feature. Do not attempt to defeat the door interlock.

> **Important:** Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

5.4. SERIES 4 INTERFACE CONTROL PANEL

The interface control panel is located on the right-hand side panel of the Series 4. It is shown in Figure 5-4.

5.4.1. MANUAL RUN/AUTO RUN Switch

In the Auto Run position this switch will inhibit the READY/RUN pushbutton on the front of the Series 4 from running the instrument. The lighted indicators on the READY/RUN pushbutton will still function. This switch puts the instrument under external control.

In the Manual Run position, the READY/RUN pushbutton on the front of the Series 4 is used to run the instrument.

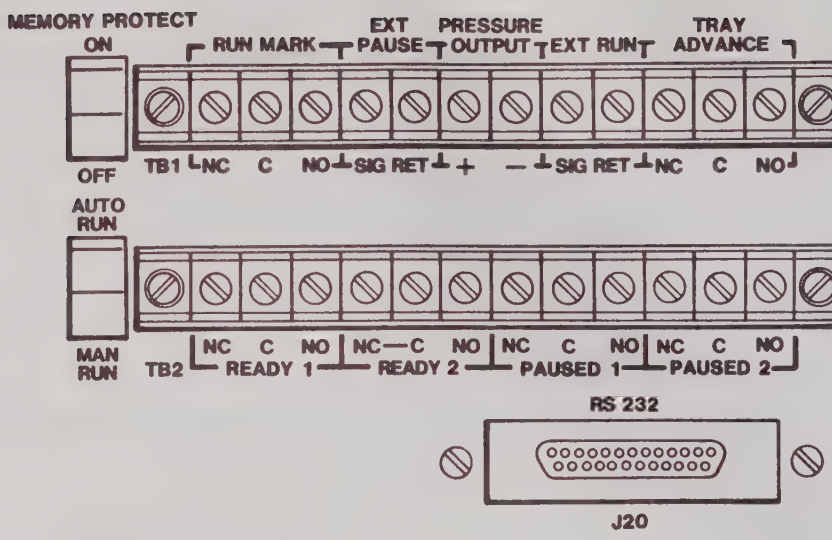


Figure 5-4 - Series 4 Interface Control Panel

5.4.2. Memory Protect Switch

In the ON position, a battery backup protects the volatile memory of the Series 4 microprocessor for 4 hours.

In the OFF position, should a power failure occur, all information stored in memory will be lost and upon power up, all information must be re-entered.

5.4.3. Input and Output Contacts

EXT RUN - INPUT - This senses a contact closure and starts the program. RUN can be triggered by the initial closure of a relay or switch.

RUN MARK - OUTPUT - This signal is an isolated contact closure which closes for approximately one second at the beginning of a chromatographic run. The signal is available as either Normally Open or Normally Closed contacts.

READY 1 AND READY 2 - OUTPUT - These signals are logically equivalent to the front panel READY lamp. They are active throughout the READY phase, but inactive at all other times. These signals indicate to an external device that the solvent system is equilibrated and ready for the next injection.

These signals are available as either Normally Open or Normally Closed contacts.

PAUSED 1 and PAUSED 2 - OUTPUTS - These signals are activated for the duration of a keyboard-commanded Pause or whenever the EXT PAUSE signal is input from an external contact closure. These signals will signify the Paused state to a stop-flow valve, 75/85 Autocontrol Detector or other external device.

EXT PAUSE - INPUT - This accepts an incoming signal from an external device to close these contacts in an isolated fashion (e.g., by relay). When these contacts are closed the pump instantly ceases pumping and all series 4 internal (software) clocks/timers will pause indefinitely. When the contacts are opened, the clocks/timers resume counting precisely, and the pump commences with previous exact flows, etc.

TRAY ADVANCE - OUTPUT - This signal is an isolated contact closure which closes for approximately one second as the program enters the EQUIL state, dependent on the chain requirements. This signal is available as either Normally Open or Normally Closed contacts. It is used for multiple sampling from a single vial.

TRAY ADVANCE - OUTPUT only works in the Chain mode.

PRESSURE OUTPUT - A voltage analog of column head pressure of 10 mv equivalent to 20 MPa is provided as a monitor and/or diagnostic.

5.5. POWER CONNECTION PANEL

5.5.1. Voltage Selection & System Fuse

A small printed circuit board located below the system fuse on the left side panel of the Series 4 must be set to correspond to the line voltage available (see Figure 5-5).

To select the proper operating voltage range setting:

(1) Remove the AC line cord from the instrument.

(2) Slide the clear plastic fuse cover all the way to the left.

(3) Rotate the FUSE PULL to the left and remove the fuse.

(4) Pull out the printed circuit board located below the fuse.

(5) Orient the printed circuit board so that the desired voltage setting is legible in accordance with the following:

AC Voltage Range	Legible P.C.B. Setting	Fuse	Part Number
90-110V	100	6.25 amp	0998-1761
105-129V	120	6.25 amp	0998-1761
180-220V	220	4 amp	0998-1616
217-158V	240	4 amp	0998-1616

(6) Push the printed circuit board back into the slot and rotate the FUSE PULL back into the normal position.

(7) Replace the fuse.

(8) Slide the clear plastic fuse cover back to the right.

9) Replace the power cord.

(10) Check to see that the POWER switch on the front of the instrument is OFF.

(11) Connect the line cord to a properly-ground AC outlet

5.5.2. Power Cord

The power cord should be connected directly to a 20A (117 VAC) or 10A (234 VAC) main power outlet with a good earth ground. The instrument should not be attached to circuits associated with equipment of a heavy-duty nature (e.g., large motors) or large or frequent loads (e.g., photo-copying systems, discharge lamps, radio transmitters).

5.5.3. Power Supply Fuse

There are two separate fuses on the Series 4 Pump Module. The system fuse is located next to the power supply cord and is explained above under VOLTAGE SELECTION. The power supply fuse is in a round fuse holder. The fuse is a 3 amp Slo-Blo fuse for 117 VAC operation (Perkin-Elmer Part No. 0993-1616). For 234 VAC operation, this fuse is a 1.5 amp. Slo-Blo (Perkin-Elmer Part No. 0998-1776).

To replace the fuse:

(1) Turn the power OFF and disconnect the power cord.

(2) Turn the fuse cap counterclockwise and remove the fuse.

(3) Replace the fuse.

If the replacment fuse also blows, contact the Perkin-Elmer Service Department.

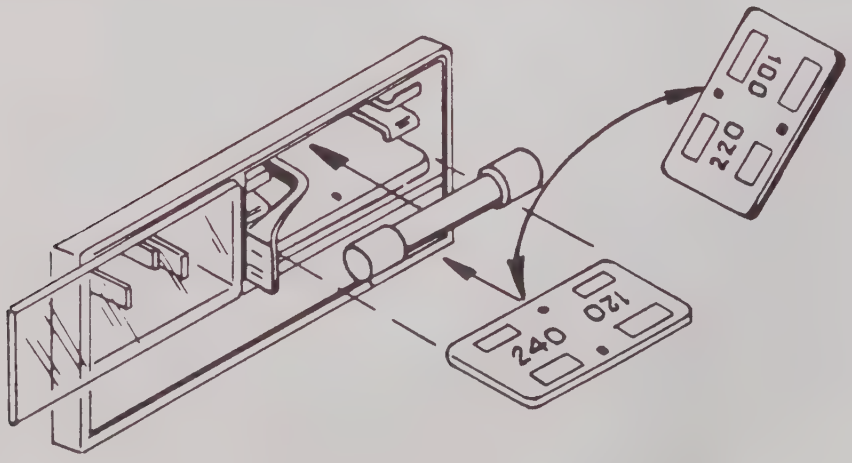


Figure 5-5 - Voltage Selection PC Board

5.6. CONTROL MODULE - FRONT PANEL CONTROLS

5.6.1. ON/OFF Switch

The ON/OFF toggle switch is located under the VDU on the right hand side of the Control Module (see Fig. 5-6)

5.6.2. Brightness Control

The brightness of the VDU may be controlled with the dial located under the VDU on the left hand side of the Control Module (See Fig. 5-6).

5.6.3. Line Key

The LINE key is shown in Figure 5-6. This key is a latching key. When depressed, it places the terminal "on-line," that is, communicating with the Series 4. When released (off-line), and with the full/half duplex switch (see Figure 5-7) in the half duplex position, the Control Module displays only data entered via the keyboard. No data are received or transmitted to the Series 4 in this position.

If there seems to be a problem with the communications between the Series 4 pump module and the Control Module, check to make sure that this key is depressed.

Figure 5-6 - Control Module (Front)

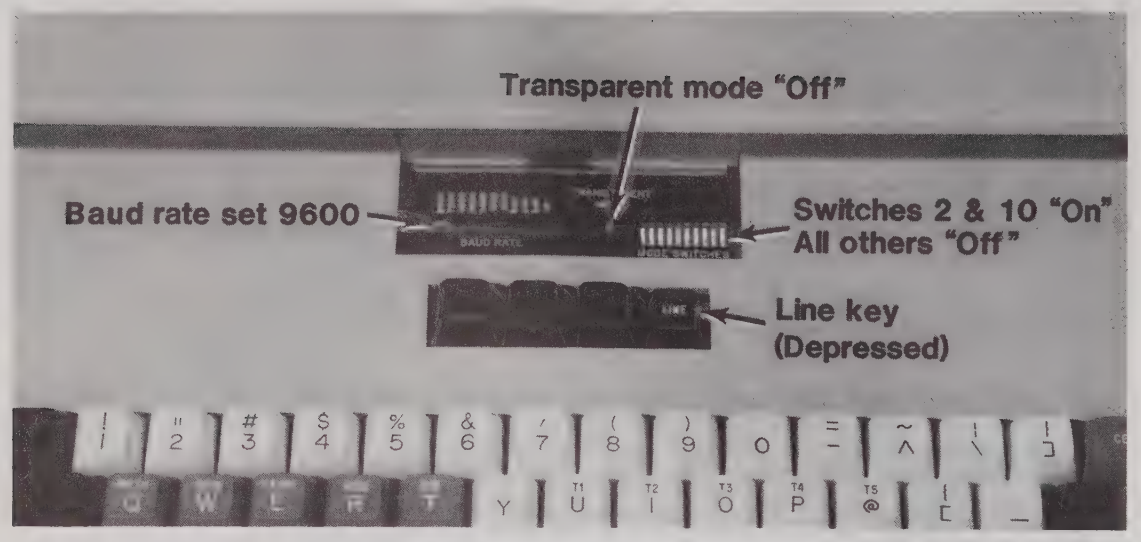


Figure 5-7 - Control Module Data Set-Up Switches

5.6.4. Data Line Set-Up

The Data Line Set-Up switches under the sliding panel above the keyboard are set at installation and should not be changed. They should be set like those shown in Figure 5-7.

SWITCH SETTINGS

Baud Rate - 9600
Transparent Mode: OFF

Parity Switches (Even Parity)

1 OFF
2 ON

Mode Switches

3 OFF
4 OFF
5 OFF
6 OFF
7 OFF
8 OFF
9 OFF
10 ON

For further information refer to the User's Manual shipped with the Series 4.

5.6.5. Setting the Parity and the Baud Rate on the Series 4 Pump Module

The Parity of the Series 4 is determined by a suitcase jumper on SP2 on the MPU board of the Series 4 Pump Module. For even parity, the jumper should be on AB. For odd parity, the jumper should be on BC. If the parity is set incorrectly, pull the jumper straight out of the pins, and carefully push onto the correct pair of pins.

The baud rate of the Series 4 is determined by a suitcase jumper on SW1 on the MPU board of the Series 4 Pump Module. To change the baud rate, carefully pull the suitcase jumper straight out off the pins, and carefully push onto the correct pair of pins. The available baud rates and the appropriate pin pairs are marked on the board.

To access the MPU board, remove the 4 screws which hold the back panel on the instrument. Look for the blue suitcase jumpers. You will see the baud rate pins near the upper left-hand corner. The parity pins are on the left side of the board about half way down.

5.7. CONTROL MODULE - REAR PANEL CONTROLS

5.7.1. RS-232 Connector Port

One end of the RS-232 cable connects to this port. The other end connects to the RS-232 port on the Interface Panel on the right hand side of the Series 4 Pump Module.

5.7.2. Printer Port

The printer port is not used.

5.7.3. Power Cord

The power cord should be connected directly to a 20A (117V) or 10A (234V) main power outlet with a good earth ground. The instrument should not be attached to circuits associated with equipment of a heavy-duty nature (e.g., large motors) or large or frequent loads (e.g., photo-copying systems, discharge lamps, radio transmitters).

5.7.4. Fuse

For 117 VAC operation, the 0.8 amp. Slo-Blo fuse is Perkin-Elmer part number 0998-1762. For 234 VAC operation, the 3/8 amp. Slo-Blo fuse is Perkin-Elmer part number 0998-1619.

(1) Turn the power OFF and disconnect the power cord.

(2) Turn the fuse cap counterclockwise and remove the fuse.

(3) Replace the fuse.

If the replacement fuse also blows, contact the Perkin-Elmer Service department.

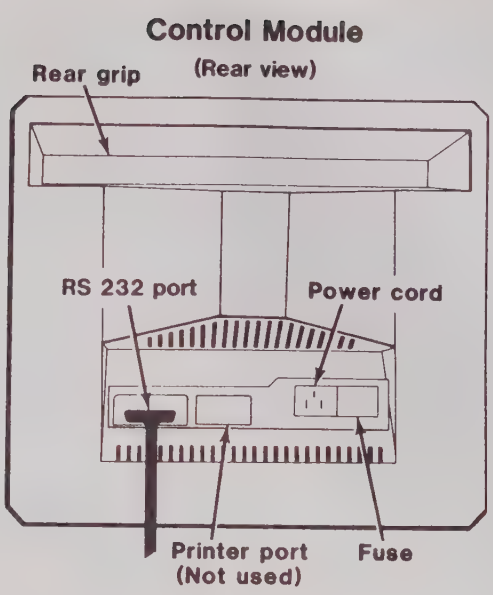


Figure 5-8 - Control Module (Rear)

5.8. CONTROL MODULE KEYBOARD

The Control Module keyboard is used to program the Series 4 Pump Module.

The functions of the individual keys are explained below. Many of the keys are dual-function keys. When used alone, they represent the alpha-numeric character; when used in conjunction with the RESET key, as will be explained below, they act as special function keys.

5.8.1. General Purpose Keys

SHIFT Key

This key permits the entry of all the upper case characters shown on the keyboard. It is a momentary action key; that is, it must be pressed each time an upper case character is desired. For an upper case character press and hold down the shift key while pressing the character key. There is no shift-lock on the Control Module keyboard .

Space Bar

This is a momentary action key; that is, it must be pressed one time for each space desired.

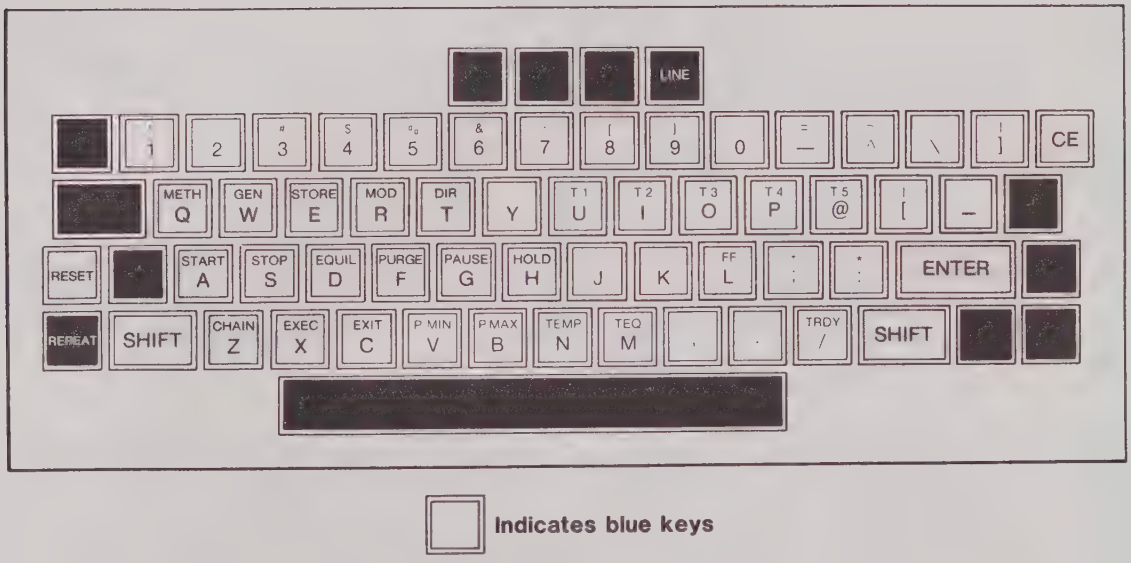


Figure 5-9 - The Series 4 Control Module Keyboard

CE Key

The CE (Clear Entry) key may be used to clear information which has been typed in before it has been entered with the ENTER key. When pressed, it will erase all information which has been typed in since the last time the ENTER key was pressed. It is used to correct or change an entry. It is also used to clear an error condition on the Series 4. The prompt will advise the user to press the CE key.

ENTER Key

The ENTER key is used to enter the information which has been typed in on the keyboard into the memory or to accept information as displayed. The ENTER key will also move the cursor to the next data entry (parameter) position.

REPEAT Key

When this key is held down while another key is pressed and held down, the function of the other key will continue to repeat until both keys are released.

Alphanumeric Character Keys

These keys are momentary action keys. When used alone, each key will write a character at the current position and move the cursor one position to the right. The cursor will stop at position 80. Any additional entries will overwrite at position 80. When the specially marked alphanumeric character keys are used in conjunction with the RESET key, they perform as special function keys as explained below.

Cursor Movement Keys

The < and > keys (used in conjunction with the SHIFT key) allow the user to move the cursor back to change previously-entered parameters or forward to a new parameter when generating or modifying a method.

The left arrow key returns the user to the beginning of the line in which the parameter occurs. the ENTER key must be used to step across the line to the correct data entry position.

5.8.2. MODE COMMAND KEYS (BLUE KEYS, UPPER ROW)

Special functions are obtained by pressing the RESET Key and holding it down while pressing the appropriate dual-function alphanumeric character key.

RESET + KEY	KEY LABEL	FUNCTION
Q	METH (method)	The METH key is used to indicate that a Method is to be generated, modified or recalled to the screen storage.
W	GEN (Generate)	The GEN special function key allows the user to generate a new method. The screen display is brought to the screen, and the cursor is positioned at the first data entry position.

RESET + KEY	KEY LABEL	FUNCTION
E	STORE	The STORE special function key stores in memory the method or Chain currently displayed on the VDU screen. The number under which the Method is stored may be selected by the user or assigned by the System. Only one Chain may be stored in memory at one time.
R	MOD (modify)	This allows the user to modify a Method. or Chain. Pressing RESET allows the user to modify the Method or Chain currently displayed on the screen. Once the modify mode has been entered, unless CE is pressed before any changes are made, all parameters must be changed or confirmed in order to exit from the Modify mode.
T	DIR (Directory)	RESET gives the user a Directory of the contents of the Series 4 memory. The Directory lists the stored Methods by number and description.

5.8.3. OPERATIONAL COMMAND KEYS (BLUE KEYS, MIDDLE ROW)

RESET + KEY	KEY LABEL	FUNCTION
A	START	This key is used to start the pump.
S	STOP	This key is used to stop the pump. If an analysis is in progress when the STOP key is used, the analysis will be aborted.
D	EQUIL (Equilibrate)	This key is used to begin the equilibration process. It will cause the system to step to the equilibration segment of the program immediately.

RESET + KEY	KEY LABEL	FUNCTION
F	PURGE	This key is used to begin the purge process. The system will immediately step to the PURGE program.
G	PAUSE	This key Pauses the system. When this key is used the Series 4 internal clock stops and the pump stops. The program will resume where it paused when the system is restarted. This is an alternating action latching key. The same key must be pressed again to restart the program, the pump and the internal clocks.
H	HOLD	This key holds the program in the current sector of a gradient analysis. The pump does not stop when this key is used. The HOLD Key is used only during gradient analyses. This is an alternating action latching key. The same key must be pressed again to restart the program.

5.8.4. CHAIN COMMAND KEYS (BLUE KEYS, LOWER ROW)

RESET + KEY	KEY LABEL	FUNCTION
Z	CHAIN	The CHAIN key is used to create an automatic sample processing sequence. Up to 100 samples may be entered in the chain, and up to 9 Methods may be specified to process the samples. Note that a Method must be in memory before it may be used in a chain.
X	EXEC (Execute)	The EXEC key is used to begin running a chain. The EXEC key will only function if the Method running is the first method in the chain.
C	EXIT	The EXIT key is used to exit from the chain sequence. It is used so that a Chain may be stopped. Once the EXIT key has been used, it is not possible to re-enter the CHAIN at the exit point. The PAUSE key should be used to temporarily stop a chain.

5.8.5. FUNCTION PARAMETER KEYS (WHITE KEYS)

RESET + KEY	KEY LABEL	FUNCTION
U	T1	This key is used in the Modify mode to step directly to the SECTOR 1 Time segment.
I	T2	This key is used in the Modify mode to step directly to the SECTOR 2 Time segment.
O	T3	This key is used in the Modify mode to step directly to the SECTOR 3 Time segment.
P	T4	This key is used in the Modify mode to step directly to the SECTOR 4 Time segment.
@	T5	This key is used in the Modify mode to step directly to the SECTOR 5 Time segment.
L	FF	This Parameter key is not used in the Series 4.
V	P MIN	This key is used in the Modify mode to enter a value for minimum pressure.
B	P MAX	This key is used in the Modify mode to enter a value for maximum pressure. If no value is entered, the default value of 42 MPa (6000 psi) will be entered
N	TEMP	The TEMP key is used in the Modify mode to enter a value for the oven temperature. It is operable only if the optional oven is connected. If a value between 10°C and 35°C is entered the temperature control will default to the OFF condition.
M	T EQ	The TEQ key is used in the Modify mode to step directly to the Equilibration Time segment.
/	T RDY	The TRDY key is used in the Modify mode to step to the Ready Time parameter entry section.

5.9. SOFTWARE-CONTROLLED PASSWORD

When turning on the Series 4 a two-character password may be entered to prevent accidental entries from affecting the analysis. If a password is entered, then it will be required to generate or modify a Method, modify a Chain or to exit from a Chain. The same password will be required until the power is turned off and back on, at which time the same password, a new password or no password may be entered.

When a password is entered, the Series 4 will momentarily print the first letter of the password on the prompt line but it will disappear when a second key is pressed. The second letter of the password will not be displayed. This helps to guard against accidental password entry. If the password is forgotten, it will be necessary to turn off power to the Series 4 and Control Module and begin again, so it is a good idea to write down the password.

When the prompt "ENTER PASSWORD" appears, the password must be entered and the command repeated before the command will be executed by the system.

If the password has been defaulted at startup by pressing the ENTER key, then all commands will be immediately effective.

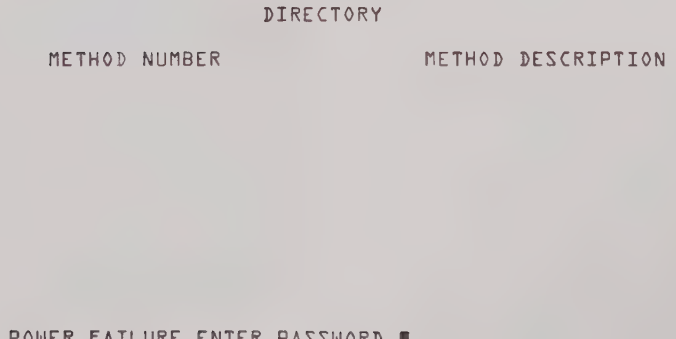


Figure 5-10 - Control Module Start Up Screen Display

5.10. SCREEN LAYOUT

The Control Module Screen Display is divided into three sections. The top part of the screen always displays the current Method. During a run The Current Status section is automatically updated so that the actual status of the pump is continuously displayed. The Current Status section is not displayed during Method or Chain Generation or Modification or while the Directory or Chain is displayed. The Prompt Line displays operator prompts and error messages.

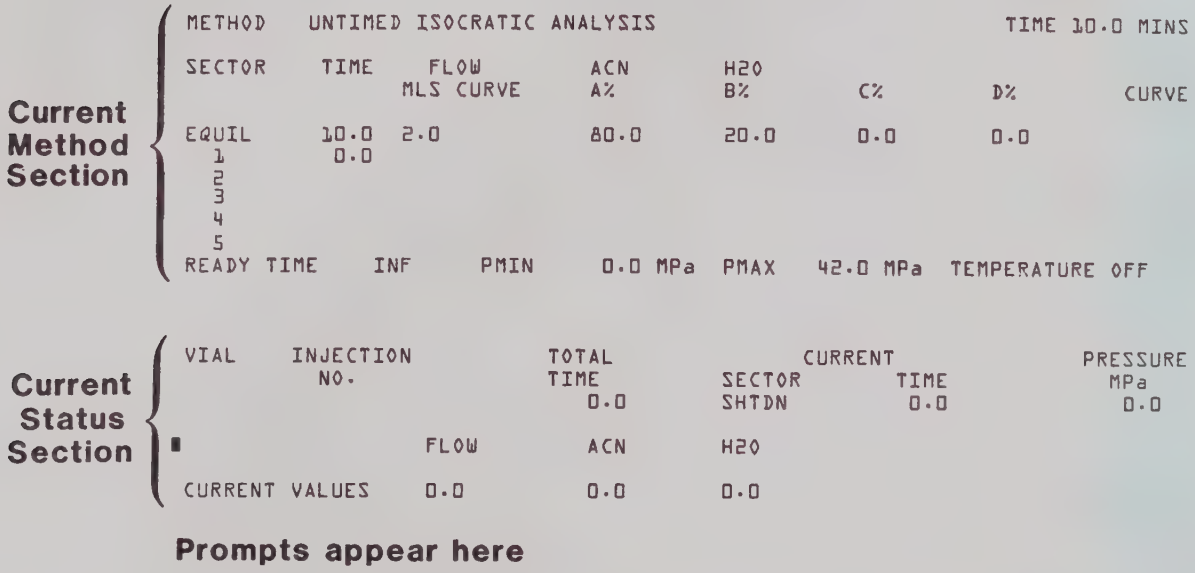


Figure 5-11 - The Control Module Screen Display Sections

6 OPERATOR AIDS

Section		Page
6.1	Operating Summary	6-1
6.2	Parameter Ranges and Default Values	6-3
6.3	Error Messages	6-4
6.4	Troubleshooting	6-5
6.5	How the Series 4 Counts Curves	6-8
6.6	Warnings and Precautions	6-10
6.6.1	Radio Frequency Energy	6-10
6.6.2	Solvents with Auto-Ignition Temperatures Below 110°C	6-10
6.6.3	Electrical Hazards	6-11
6.6.4	Mechanical Hazards	6-11
6.6.5	Corrosion	6-12
6.6.6	Pressure Build-Up	6-12
6.6.7	Air Bubbles	6-12
6.6.8	Buffers	6-13
6.6.9	Solvents	6-13
6.6.10	Solvents with Low Boiling Points	6-13
6.6.11	Magnetic Door Latch	6-13
6.6.12	Helium Supply	6-13
6.7	Blank Method Pages	6-14

SERIES 4 LIQUID CHROMATOGRAPH
OPERATING SUMMARY

START UP

(1) Turn the power ON at the Control Module and then at the Pump Module. The switch on the Pump Module will light, the Control Module will "beep" and the prompt will say:

POWER FAILURE ENTER PASSWORD?

(2) Enter a password or default the password by pressing the ENTER key.

GENERATING A METHOD

(1) Press the RESET key and hold it down while pressing the GEN key.

(2) Enter the required parameters at each data entry position. When entries are complete, the cursor will flash in the current values section of the screen display.

PRIMING THE PUMP & PURGING THE SYSTEM

(1) Connect the priming syringe to the flush port.

(2) Press the RESET key and hold it down while pressing the PURGE key. The program will step immediately to the program. The prompt will ask which reservoir is to be used to purge.

(3) Type in the reservoir (A,B,C, or D) to be used to purge.

NOTE: The reservoir to be used to purge must be identified in the Method

(4) In response to the flow rate query, press the ENTER Key to get the default purge flow rate of 0 (zero). The valve will audibly click open.

(5) Open the drain valve by turning the handle counterclockwise.

(6) Collect about 20 milliliters of solvent in the syringe or continue until bubbles no longer are being expelled. Make sure that the drain valve is fully closed before removing the priming syringe.

(7) Press the RESET key and hold it down while pressing the STOP key to exit from the purge program.

RUNNING A METHOD (STARTING THE PUMP)

(1) The Method to be run must be displayed on the screen. See "Recalling a Stored Method", below.

(2) On the Control Module, press the RESET key and hold it down while pressing the START key. This will start the pump. When the equilibration time has passed, the READY button will illuminate.

(3) On the Pump Module, press the RUN button when the system is stable as indicated by an acceptable detector baseline.

NOTE: Since an untimed isocrated method runs continuously, the RUN button is disabled in this mode.

STOPPING A RUNNING METHOD (STOPPING THE PUMP)

(1) Press the RESET key and hold it down while pressing the STOP key on the Control Module. This will abort the analysis.

STORING A METHOD

(1) Press the RESET key and hold it down while pressing the STORE key.

(2) The screen prompt will request:

ENTER METHOD STORAGE NUMBER?

(3a) Pressing the ENTER key will cause the system to assign the next available number to the method to be stored.

(3b) Entering a number which has already been used to store a previous Method or if no storage number is available will cause the prompt to say:

OVERWRITE EXISTING METHOD (Y/N)?

(3c) If the Method to be overwritten has been used in a Chain, the prompt will say:

METHOD EXISTS IN CHAIN, OVERWRITE (Y/N)?

(4a) Answering N will exit from the Store program. Go back to step (2) and assign a different number or use the ENTER key to assign the next available number.

(4b) Answering Y will replace the Method stored under that number with the method presently shown on the screen display.

NOTE: Since Untimed Isocratic Methods cannot be used in a Chain, a Method with an infinite ready time cannot replace a Timed Isocratic Method if the Method has been used in a chain. The prompt will say UNTIMED ISOCRATIC METHODS ARE NOT ALLOWED IN CHAIN. Assign a new storage number

RECALLING A STORED METHOD TO THE SCREEN

The Method displayed on the screen must be in the shutdown sector to be replaced by a Method from storage

(1) Recall the stored Method to the screen by pressing the RESET key and holding it down while pressing the METH key on the Control Module. The prompt will ask for the Method number.

(2) Type in the number of the Method to be called to the screen from storage and press the ENTER key.

MODIFYING AN EXISTING METHOD

(1) The Method to be modified must be displayed on the Control Module. If it is not presently displayed, call the Method to the screen before continuing with the instructions below.

NOTE: A Method may be modified only when the Pump is in EQUIL or SHUTDOWN. Step to the EQUIL Sector using the RESET key and the blue EQUIL key

(2) Press the RESET key and hold it down while pressing the MOD key on the Control Module. The prompt will say

SELECT SECTOR OR PRESS ENTER KEY

(3a) Press the appropriate parameter key (e.g., T1, T5, etc.) to step directly to the time sector where the parameter to be changed is located; or

(3b) Press the ENTER key once to indicate to the Series 4 that the ENTER key will be used to step through the parameters. The Series 4 will be in the Method description data entry position. Enter a new description or default the entry by pressing the ENTER Key. The Series 4 will step to the next data entry position.

(4) When modifications are complete, use the REPEAT and ENTER keys to step through the remaining parameters and exit from the Modify mode.

(5) Store the modified Method.

(6) If the Method has been used in a Chain, when the user attempts to store the modified Method, the prompt will say:

METHOD USED IN CHAIN, OVERWRITE (Y/N)?

(6a) Type Y to store the modified Method. If a Method used in a Chain is modified, the modifications will subsequently be used in the Chain. Check to make sure modifications will be appropriate in the Chain.

(6b) Type N to preserve the unmodified Method. Answering N exits from the store routine. Repeat the Store Procedure, using a different storage number for the modified Method.

OBTAINING A DIRECTORY OF MEMORY CONTENTS

(1) Press the RESET key and hold it down while pressing the DIR key. The Method screen display will be replaced by the Directory.

(2) To return to the previously displayed Method press the RESET key and hold it down while pressing the METH key. The prompt will say:

CLEAR CURRENT METHOD (Y/N)?

N will return the previously displayed Method to the screen. Y will cause a Method number request.

(3) Type in the number of the Method to be displayed.

NOTE: If you are in the process of generating or modifying a method you must complete the generation or modification before calling the Directory

CREATING A CHAIN

(1) Press the RESET key and hold it down while pressing the GEN key on the Control Module. The prompt will say:

PRESS METHOD, CHAIN OR CE

(2) Press the CHAIN key. The Chain screen will appear.

(3) Enter the sample parameters and corresponding Methods.

NOTE: T Ready - INF must not be used in any Method to be chained

(4) When all entries have been made, press the RESET key and hold it down while pressing the STORE key.

MODIFYING A CHAIN

(1) Press the RESET key and hold it down while pressing the CHAIN key. This will display the existing chain.

(2) Press the RESET key and hold it down while pressing the MOD key. The prompt will read:

ENTER FIRST VIAL NUMBER OF LINE TO BE MODIFIED

(3) Type in the requested number. The cursor will step to the line to be changed. Use the ENTER key to step to the proper data entry position, type in the new value and press the ENTER key as many times as needed to leave the line. When modifying a line, the left arrow key may be used to step back to the beginning of the line being modified

The change will be stored automatically.

To make another modification at this time, repeat steps (2) and (3) above.

To exit from the program.

PRESS RESET 'METH or RESET 'DIR

RUNNING A CHAIN

(1) The first Method in the chain must be displayed. If it is not, call the first Method to the screen before continuing.

(2) Press the RESET key and hold it down while pressing the START key to start the pump.

(3) Press the RESET key and hold it down while pressing the EXEC key to start the chain

(4) When the Equilibration time has run, the READY Light on the Pump Module will light. Press the RUN key on the Pump Module

NOTE: The Pump Module must receive a RUN signal from an external source (such as the Autosampler) or the RUN button must be pressed for EACH analysis in the chain.

STOPPING A CHAIN RUN

(1) Press the RESET key and hold it down while pressing the EXIT key. The prompt will read: CHAIN EXIT (Y/N)? N will cancel the exit request. Type Y to exit.

(2) Press the RESET key and hold it down while pressing the STOP key. This will abort the analysis in progress, if the Chain has stopped.

NOTE: If a Chain is stopped before completion, the entire chain must be rerun or the Chain must be modified to delete the Methods which are not to be rerun. It is not possible to exit from a Chain and subsequently re-enter the Chain at the exit point.

If a Chain should be stopped temporarily, the PAUSE key, rather than the EXIT key should be used.

NOTE: The HOLD and Pause keys are latching keys. The same key must be pressed to restart the system as was used to stop it.

USING THE HOLD AND PAUSE KEYS

(1) Press the RESET key and hold it down while pressing the HOLD or PAUSE key. The PAUSE key will pause the pump and the internal clocks on the Series 4. The HOLD key will stop the internal clocks, but the pump will continue to run.

(2) To continue the analysis, press the RESET key and hold it down while pressing the same HOLD or PAUSE key again. This will restart the pump and/or the internal clocks on the Series 4.

USING THE COLUMN OVEN

When the column oven is connected, the TEMPERATURE parameter will be requested when the Series 4 Method is generated. If this parameter is defaulted, the temperature control will be OFF. If the oven is to be used, a value between 35°C and 99°C should be entered in the Method. If the value entered is between 10°C and 34°C, the temperature control will default to a power OFF condition.

TIMED MODES

When a timed run is programmed with a heated column, after the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

CAUTION: The cool-down sequence protects the column Do not accidently use the STOP key to stop the pump during the cool-down sequence

UNTIMED ISOCRATIC MODE

If a heated oven is used in an untimed isocratic run a cool-down must be provided by modifying the Method without stopping the pump.

(1) Press the RESET key and hold it down while pressing the EQUIL key. This will step to the equilibration sector.

CAUTION: DO NOT STEP TO THE SHUTDOWN SECTOR TO MODIFY THE TEMPERATURE

(2) Press the RESET key and hold it down while pressing the MODIFY key

(3) Press the TEMP key to step directly to the temperature parameter.

(4) Type 10 or any value between 10 and 34 and press the ENTER key. This will turn the oven OFF.

(5) Allow the solvent to flow for a sufficient period of time to cool the column or remove the column to protect it before using the STOP key to stop the pump.

CHAINING METHODS WHICH USE THE OVEN

All Methods which do not use the oven temperature control should precede those which use temperatures between 35°C and 99°C. Methods which use the oven should be arranged so that the temperature increases as the Chain progresses

EMERGENCY SHUTDOWN PROCEDURE

(1) Turn off power to the pump and control modules.
(2) Open the door to the solvent chamber.
(3) Shut off the helium supply at the helium tank.
(4) Open the oven doors.

6.2. PARAMETER RANGES AND DEFAULT VALUES

PARAMETER	RANGE	DEFAULT VALUE
METHOD DESCRIPTION	40 alpha-numberic characters	None
SOLVENT RESERVOIR IDENTIFICATION	up to 5 alpha-numeric characters	None
EQUIL TIME	0.1 - 999.9 mins.	10 minutes
FLOW - MLS	0.1 - 9.9 ml/min.	2.0 ml/min.
FLOW - CURVE	0.5 - 5.0	0 (zero is a step function)
PERCENT SOLVENT RESERVOIRS A-D	0.0 - 100.0% for each*	None (% SOLVENT Must always be entered)
	*Total must not exceed 100%	
EQUIL TIME	0.0 - 999.9 min.	10 min.
Sector 1-5 TIME	0.0 - 999.9 mins.	0 (zero)
EQUIL FLOW - MLS	0.1 - 9.9 ml/min.	2.0 ml/min.
Sector 1-5 FLOW - MLS	0.1 - 9.9 ml/min.	2.0 ml/min.
Sector 1-5 FLOW - CURVE	0.5 - 5.0	0 (zero is a step function)
SECTOR 1-5 PERCENT SOLVENT RESERVOIRS A-D	0.0 - 100.0% for each*	None (% SOLVENT Must always be entered)
	*Total must not exceed 100%	
READY TIME	0.0 - 999.9 mins.	INFINITE if 0 is entered in Sector 1 TIME (untimed isocratic analysis); 60.0 if a value greater than 0 is entered in Sector 1 TIME (timed isocratic analysis or gradient or flow program analysis).
PMIN	0 - 41.9 MPa*	0 MPa.
PMAX	0.1 - 42 MPa*	42.0 MPa
	*PMIN must be always less than PMAX.	
TEMPERATURE	35°C - 99°C	OFF

6.3. ERROR MESSAGES

Note: An Asterisk (*) indicates that an audible signal accompanies the message.

MESSAGE	EXPLANATION	SOLUTION
ENTER PASSWORD	If a password is entered upon power up, this same password will be required to Generate or Modify a Method, to Modify a Chain or to Exit from a Chain.	Enter password. If you have forgotten it, turn off power to both the Series 4 Pump Module and the Control Module and turn it back on. Re-enter password or default entry.
INCORRECT PASSWORD	The wrong 2-character password was entered.	Re-enter the password; Turn power off and back on as above.
*ERROR, CE	The last parameter entered was out of range or in an incorrect form.	Press the CE key and type in the correct parameter. If a default value is to be entered, it must be typed in as merely pressing the ENTER key will re-enter the incorrect parameter again.
*TOTAL PERCENT EXCEEDS 100%	The percentages to be taken from reservoirs named in Method exceed 100% when totaled.	Re-enter the correct percentages to be used from each identified solvent reservoir.
*UNTIMED ISOCRATIC METHODS ARE NOT ALLOWED IN CHAIN	The Method to be used in Chain has an infinite Ready Time	Modify the Method so that the Ready Time is between 0.1 and 999.9.
*METHOD DOES NOT EXIST, CE	The Method to be recalled to the screen is not stored under the number given.	Re-enter the Method number. If you don't know the number the Method was stored under, press the RESET key and hold it down while pressing the DIR key to obtain a DIRECTORY of Memory contents. Then press the RESET key and hold it down while pressing the METH key. Enter the correct Method number when requested by the prompt.
*MODIFY METHOD IN EQUIL OR SHUTDOWN ONLY	The Method is "Ready" or "Running".	Press the RESET key and hold it down while pressing the blue EQUIL key. The Series 4 will step immediately to the EQUIL Sector. Modify as necessary.
*PMIN GREATER THAN OR EQUAL TO PMAX	The value entered for PMIN equals or exceeds PMAX.	Press the CE key. Then type in a value for PMAX which exceeds PMIN. If necessary, use the shift key and the left arrow key to return to the beginning of the line. Then press the ENTER key to step to the PMIN data entry position. Re-enter the value for PMIN. Then re-enter the value for PMAX.
*TOO MANY CURVES	Only two curves may be entered in each time sector.	Re-enter the parameters to eliminate the extra curves. Asterisks will indicate each parameter to be altered.

6.4. TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Communications Problems		
No Display at all	Terminal unplugged, power switch off, brightness setting too low; blown fuse.	Check plug; check to see that Control Module ON/OFF switch is in the ON position; turn up the brightness control and then check for blown fuse.
No communication between Control Module and Series 4 Pump Module (RESET/START does nothing, for example.)	RS-232 Cable not connected; Line key not depressed.	Check to see that LINE key is depressed; check to see that RS-232 cable is connected properly.
Garbled Screen when LINE key depressed. When LINE key is up, Control Module operates properly.	Baud rate improperly set. Set Baud rate at 9600.	Refer to Section 5, Fig. 5-7, and set the switches correctly on the Control Module. Then check the jumpers on the Pump Module MPU board.
Every other character is displayed	Full/Half Duplex switch set to FULL	Set Full/Half Duplex switch to HALF.
Screen too light/dark	Brightness control is improperly set	Adjust brightness control.
Control Module fuse blows after replacement		Call Perkin-Elmer LC Service Department
Parameter Entry Problems:		
Forgot Password	If a password is entered upon power up, this same password will be required to Generator or Modify a Method, to Modify a Chain or to Exit from a Chain.	If you have forgotten the password, turn off power to both the Series 4 Pump Module and the Control Module and turn it back on. (Memory Protect Switch must be off.) Re-enter password or default entry.
OFF/ON procedure described above does not clear password request.	Memory Protect Switch is in the ON position.	Turn the Memory Protect Switch off. Repeat the OFF/ON procedure. Turn the Memory Protect switch back on.
Control Module beeps	Read prompt line	Follow directions of prompt or reread the instructions for parameter entry.
Passed data entry position accidentally		Hold down the shift key and press the left arrow key until the cursor has returned to the beginning of the line which contains the data entry position. Use the ENTER key to step across the line to the proper position. Enter the parameter.
Previously-entered parameters in Sectors 1-5 are erased during Method generation or modification.	A ready time of zero has been entered.	Use the shift key and the left arrow key to return to the appropriate lines and re-enter the parameters. Enter a ready time between 0.1 and 999.9.
Won't pass data entry line	Incorrect parameter entries have been made.	Follow directions of prompt or reread instructions for parameter entry. Re-enter values within range.
Improper TIME entry	Attempted to enter % for last named reservoir.	Use the shift key and the left arrow key to return to the data entry position. Type in the correct value and press the ENTER key.

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Re-entered value and got the wrong value again.	Attempted to enter a default value by just pressing the ENTER key after clearing an error.	The correct value must be typed in following a "clear entry" as pressing the ENTER key will re-enter the error from buffer memory rather than entering the default value.
Last solvent percentage not computed automatically.	The last solvent to be used is not the last identified reservoir.	Use the shift and left arrow keys to return to the beginning of the line to be modified. Step across to the proper position using the ENTER key. Type in the last solvent percentage and press the ENTER key.
Command Problems:		
System does not respond to command keys	The RESET and FUNCTION keys are notsimultaneous action keys.	Press the RESET key and hold it down while pressing the FUNCTION key.
System will not START	System is still in the Generate, Modify or Purge Mode.	If the system is still in the Generate or Modify Mode, finish stepping through the parameter entries to exit. RESET/STOP must be used to exit from the Purge Mode.
CHAIN won't run	The first Method in the Chain must be displayed on the LC Terminal and must be started before the Chain is executed.	Start the first Method in the chain running. Then press the RESET key and hold it down while pressing the EXEC key.
System does not step to the EQUIL Sector	Used white TEQ key instead of blue EQUIL key.	Press the RESET key and hold it down while pressing the blue EQUIL key.
System won't restart after HOLD or PAUSE	The HOLD and PAUSE keys are latching keys. The same keys must be used both to HOLD (PAUSE) the system and to restart it.	To restart the system after a HOLD (PAUSE), press the RESET key and hold it down while pressing the HOLD (PAUSE) key which was used to hold or pause the system.
Pump does not stop automatically after analysis	If the Ready Time entered in the Method is INFINITE, the pump will run until it is stopped by the operator or by an automatic safety function (e.g., the pressure drops below PMIN).	Modify the Method to make it a Timed Isocratic Analysis.
Can't recall METHOD from storage.	Method was not stored.	
Can't re-enter Chain at exit point	The first Method in the Chain must be running when the Chain is executed.	Chain must be modified so that Method re-entry point is the first Method in the Chain. Rerun Chain.
Operation Problems:		
Pump shuts down automatically	Pressure dropped below PMIN or exceeded PMAX	Check for empty solvent reservoir; check for leak if pressure drops below PMIN. If PMAX exceeded, clean injector, replace intake filter, column end fitting or column.
Leaky seals or check valve failure	salt crystals from buffer left in system.	Replace seals and/or check valves. Do not leave buffers in system.

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
pump flow fluctuation	restriction in intake filter	Clean or replace intake filter
READY/RUN button inoperable	Method is an untimed isocratic method; AUTO/MANUAL Run switch set for external control.	READY/RUN button is inhibited for untimed isocratic method; set AUTO/MANUAL run switch to MANUAL Position.
Oven does not heat	Temperature entered in Method less than 35°C; oven overheated, cycling safety switch.	Enter a temperature between 35°C and 99°C. Call your Perkin-Elmer LC Service Representative.
Pressurization Problems:		
Solvents won't pressurize	Helium supply exhausted; helium not reaching solvent; o-ring on solvent reservoirs missing or improperly seated; door is not latched properly; door interlock is defective	Replace helium tank; check helium supply at inlet fitting; check for restriction in tubing; check output at regulator; check that regulator is properly seated in tank. Replace regulator if defective. Reseat or replace o-ring(s) and recap solvent reservoirs; reclose door to solvent pressurization chamber; call your Perkin-Elmer Service Representative.
Sparging continues after door to pressurization chamber is closed	O-ring(s) on solvent reservoirs improperly seated or missing; leak in system.	Reseat or replace o-ring(s) and recap solvent reservoirs; check all fittings for leaks.
Air Bubbles	intake filters above solvent level in reservoirs; insufficient priming of pump.	Make sure that intake filters are below the solvent level in the reservoirs; step to the Purge Mode and repeat the Purge procedure until no air bubbles are coming through the system.
Solvent escapes at helium input fitting	Helium input check valve failure	Replace the helium input check valve.
Solvents cross-contaminating	Feedback loop check valve failure	Replace the feedback loop check valve.
Miscellaneous Problems:		
Did not get upper case characters (capital letters)	There is no shift lock on the Series 4 keyboard.	Press the SHIFT key and hold it down while pressing the key which is to be upper case.
Solvent reservoirs have inadequate capacity for analysis to be done.		If a two-solvent analysis is to be run, all four reservoirs can be identified, and two filled with each solvent. (See Section 4.16.)
Tray Advance Output does not work	Tray Advance Output only works when the Series 4 is in the CHAIN Mode.	To use an Autosampler, a dummy chain should be created. See instructions for creating a Chain.

6.5. HOW THE SERIES 4 COUNTS CURVES

The Series 4 has five different independently-programmable time sectors. For each of these time sectors, the user can specify time, flow rate and flow curve, percent composition and a gradient curve if a gradient is to be used. A gradient curve is a smooth transition from one percent solvent level in a time sector to another percent solvent level in the succeeding time sector. A flow curve is a smooth transition from one flow rate in a time sector to another flow rate in the succeeding time sector. Two curves are allowed in each sector; one flow rate change and one solvent gradient or two solvent gradients.

For example, if the flow rate varies, then only one solvent curve can be run. If the flow rate is held constant, then two solvent curves can be run, the third solvent can be held at same constant value and the fourth is the balance or complement.

Since the last identified reservoir is always used to make up the residual to insure that the total percentages always equal 100%, a change in solvent composition in this last reservoir is therefore not counted as a "curve".

For example, if two reservoirs are identifed, and a flow curve is to be used with the gradient curve, the change in the last identified reservoir (Reservoir B) is not counted as a curve since it is used to make up the residual to insure that the total percentages equal 100% as shown below.

A

SECTOR	TIME	FLOW MLS	CURVE	ACN A%	H2O B%	C%	D%	CURVE
EQUIL	2.0	2.0		80.0	20.0			
1	2.0	1.0	1.0	20.0	80.0			
2								
3								
4								
5								

However, if four reservoirs were identified, the last identified reservoir would not be the one being used to make up the residual. Hence, the Method below would not be acceptable and the prompt would read: TOO MANY CURVES.

B

SECTOR	TIME	FLOW MLS	CURVE	ACN A%	H2O B%	MEOH C%	THF D%	CURVE
EQUIL	2.0	2.0		90.0	10.0	0.0	0.0	
1	2.0	3.0*	1.0	10.0*	90.0*			1.0
2								
3								
4								
5								

If only one solvent curve is to be run in a sector, the last identified reservoir must be used to balance the curve.

If, for example, four reservoirs are identified and a flow curve is to be run in a sector, then only one solvent change may occur in the first three identified reservoirs and the balancing solvent change must be made in the fourth reservoir.

In the example below, there are 2 solvent curves.

C

SECTOR	TIME	FLOW MLS	CURVE	ACN A%	MEOH B%	THF C%	H2O D%	CURVE
EQUIL	2.0	2.0		65.0	2.0	5.0	28.0	
1	2.0	2.0		2.0	65.0	5.0	28.0	1.0
2								
3								
4								
5								

In the example below, there are also 2 curves--one flow curve and one solvent curve. The change in the last reservoir, from 28% to 20% is not considered a curve since the last identified reservoir is being used to make up the residual.

D

SECTOR	TIME	FLOW MLS	CURVE	ACN A%	MEOH B%	THF C%	H2O D%	CURVE
EQUIL	2.0	2.0		2.0	65.0	5.0	28.0	
1	2.0	3.0	1.0	10.0	65.0	5.0	20.0	1.0
2								
3								
4								
5								

However, in this example, there are 3 curves: one flow curve and two solvent curves.

E

SECTOR	TIME	FLOW MLS	CURVE	ACN A%	MEOH B%	THF C%	H2O D%	CURVE
EQUIL	2.0	2.0		2.0	65.0	5.0	28.0	
1	2.0	3.0*	1.0	10.0*	57.0*	5.0	28.0	1.0
2								
3								
4								
5								

Because the last identified reservoir is always used to balance the curve, it is not advisable to identify reservoirs which will not be used in a Gradient or Flow Program Method.

The accompanying examples illustrate permissible entries and illegal entries.

The Series 4 will not allow you to enter too many curves in a time sector. If you attempt to do so, the prompt line will read:

TOO MANY CURVES

and asterisks will appear on the screen next to each entered curve. The cursor will return to the beginning of the line in which the incorrect entries were made.

If you need to go back to change earlier entries, use the shift key and left arrow key to return to the beginning of the line in which the changes are to be made.

6.6. WARNINGS AND PRECAUTIONS

6.6.1. Radio Frequency Energy

The Series 4 generates, uses and can radiate radio frequency energy, and, if not installed and used in accordance with this instruction manual, may cause interference with radio communications. It has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC Rules, which are designed to provide reasonable protection against such interference when operated in a commercial environment.

Operation of this equipment in a residential area is likely to cause interference, in which case the user, at his own expense, will be required to take whatever measures may be required to correct the interference.

6.6.2. Solvents With Auto-Ignition Temperatures Below 110°C

Certain solvents have a temperature at which they are combustible upon contact with a heated surface even in the absence of a spark or other source of ignition. For your convenience, a list of common solvents with their auto-ignition temperatures is given below.

Warning: DO NOT USE CARBON DISULFIDE OR OTHER SOLVENTS WITH AN AUTO-IGNITION TEMPERATURE BELOW 110°C.

AUTO-IGNITION TEMPERATURES OF COMMON LC SOLVENTS

SOLVENT	AUTO-IGNITION °C
pentane	309
hexane	261
isooctane	418
petroleum ether (naptha)	288
cyclohexane	260
i-propyl ether	443
diethyl ether	180
benzene	562
THF	321
methylethylketone	474
acetone	538
acetonitrile	524
ethanol	423
methanol	446
propanol	404
carbon disulfide	100
dioxane	366
dimethylformamide	445
toluene	536
ethyl acetate	524
isopropanol	455

6.6.3. Electrical Hazards

Warning: High voltage is present at the power supply PC board in the Series 4 Solvent Delivery System. Before removing any panels, the Series 4 must be turned OFF and disconnected from the AC power source.

Electrical service on the Series 4 Solvent Delivery System should be performed only by a qualified Perkin-Elmer Service Engineer.

Normal maintenance on the pump module such as replacement of seals or check valves can be performed by the user. See the Service and Maintenance Section for instructions. Do not pull out the pump module until the Series 4 has been disconnected from the AC power supply.

6.6.4. Mechanical Hazards

Warning: The Series 4 Pump Module contains moving parts that could cause operator injury. Before attempting to perform routine maintenance or service, make sure that all motion is stopped and that the Series 4 is turned off and disconnected from the AC power source.

6.6.5. Corrosion

All portions of the instrument which come in direct contact with the solvent are made of 316 stainless steel (or equivalent), PTFE, glass, gold/nickel alloy, or sapphire. Some of these materials are extremely sensitive to acidic chlorides.

> **Important:** Do not permit acidic chlorides (those with pH less than or equal to 7) to come in contact with any of the internal portions of the instrument. Instrument corrosion or failure resulting from the use of acidic chlorides is NOT covered by the warranty.

For example, some of the internal portions of the instrument are extremely sensitive to hydrochloric acid. Several publications have described the use of "TRIZMA" buffers in ion exchange work. These are available in several forms. The acid form tris (hydroxymethyl) aminomethane HCl (TRIS-HCl), contains hydrochloric acid and MUST NOT BE USED. The pH conditions required to duplicate most published separations can be met with the phosphoric version, Mono-Tris (Hydroxymethyl) aminomethane phosphate (TRIS-H_3PO_4).

In addition, some users of LC systems have observed that chloroform and carbon tetrachloride, although not ionized themselves, slowly decompose to liberate hydrochloric acid. HCl, as noted above, attacks stainless steel. Do not leave these solvents in the system for a prolonged period if the system is not being used. Should corrosion occur, thoroughly flush the entire system with methanol and then with water. Then call your Perkin-Elmer Service representative to receive instructions on re-passivating your system.

6.6.6. Pressure Build-Up

Excessive solvent pressures at the pump outlet which could shut down the system may occur. If pressures in excess of 42 MPa (6000 psi) are noted, or if pressures greater than 3.45 MPa (500 psi) above the normal operating pressures for a given procedure are observed, proceed as follows:

(1) If "clean" samples have been used, and no other apparent cause for the pressure build-up is probable, remove the injector and clean it.

(2) If "dirty" samples have been used, the injector may be clogged or the column packing materials may have retained contaminated material. Remove the injector, clean it, and replace the column.

(3) The column end fitting may have become clogged. Replace the end fitting to relieve excess pressure.

6.6.7. Air Bubbles

In order to prevent air from entering the system and to ensure that pressure fluctuation does not occur, the following precautions should be observed.

Always make certain that the intake filter is kept below the solvent level in the solvent reservoir.

When priming the system after the instrument has not been used for a period of time, remove all the air bubbles by fast-flushing the system or by venting sufficient liquid as explained in the Operation Section (Purging the Pump). After priming, close the drain valve completely.

6.6.8. Buffers

Be careful when using buffers in conjunction with organic solvents. Never leave buffers in the Series 4 Solvent Delivery System when it is not running. Salt crystals can form, destroying seals and causing check valve failure.

6.6.9. Solvents

Use clean solvents. Solvents which have been distilled in glass are highly recommended.

Degas all aqueous and most organic solvents prior to use.

Carefully use and store flammable solvents which may form hazardous by-products when the instrument is shut down.

Check compatibility of solvent(s) with the type of column(s) being used.

Know the relative polarity and miscibility of the solvents being used for analysis.

6.6.10. Solvents With Low Boiling Points

Any liquid with a boiling point less than or equal to 50°C should be used under pressurization in the solvent chamber to reduce the incidence of bubble formation in the pump inlet.

Warning: Do not use carbon disulfilde or other solvents with an auto-ignition temperature below 110°C.

6.6.11. Magnetic Door Latch

Important: Do not place magnetic tapes or computer discs near the magnetic door latch on the Series 4 Pump Module.

6.6.12. Helium Supply

Important: Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

Important: Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before any period of prolonged shutdown.

Important: Do not exceed 100 psi.

6.7. BLANK METHOD PAGES

In this Section we have provided blank Screen Display pages both for individual Methods and Chained Methods.

We suggest that you make Xerox copies of these "master pages" and use them to record the parameters of important Methods to be saved.

NO. OF SAMPLES	VIAL NO. F	VIAL NO. L	NO. OF INJECT	METHOD	METHOD DESCRIPTION

METHOD _____ TIME _____ MINS

SECTOR	TIME	FLOW MLS	FLOW CURVE	A%	B%	C%	D%	CURVE
EQUIL	____	___	__	____	____	____	____	__
1	____	___	__	____	____	____	____	__
2	____	___	__	____	____	____	____	__
3	____	___	__	____	____	____	____	__
4	____	___	__	____	____	____	____	__
5	____	___	__	____	____	____	____	__

READY TIME _____ PMIN _____ MPa PMAX _____ MPa TEMPERATURE _____

7 FAMILIARIZATION EXERCISES

Section		Page
7.1	Materials Needed	7-1
7.2	The Series 4 Method	7-1
7.3	Untimed Isocratic Analysis	7-2
7.3.1	Generating an Untimed Isocratic Analysis	7-2
7.3.2	Storing the Untimed Isocratic Method	7-7
7.3.3	Recalling the Method from Storage	7-8
7.3.4	Degassing (Sparging) the Solvents	7-8
7.3.5	Pressurizing the Solvents	7-9
7.3.6	Priming the Pump and Purging the System	7-9
7.3.7	Running the Untimed Isocratic Method	7-11
7.3.8	Modifying the Untimed Isocratic Method	7-12
7.3.9	Stopping the Pump	7-15
7.4	Timed Isocratic Analysis	7-16
7.4.1	Modifying an Existing Method	7-16
7.4.2	Storing the Timed Isocratic Method	7-17
7.4.3	Running the Timed Isocratic Method	7-18
7.5	Gradient Analysis	7-19
7.5.1	Generating a Gradient Method	7-21
7.5.2	Storing the Gradient Method	7-26
7.5.3	Running the Gradient Analysis	7-26
7.5.4	Using the Hold and Pause Keys	7-28
7.5.5	Stopping the Gradient Analysis	7-29
7.6	Obtaining A Directory Of Memory Contents	7-29
7.7	Chaining Methods Together For Unattended Analyses	7-30
7.7.1	Generating a Chain	7-30
7.7.2	Storing a Chain	7-32
7.7.3	Running a Chain	7-32
7.7.4	Leaving the Chain	7-33

7. FAMILIARIZATION EXERCISES

The purpose of these familiarization exercises is not to provide exhaustive information on the controls and parameter entry requirements (detailed information is given the Controls and Indicators and Operation Sections of this manual). Rather, this exercise uses a C8 5 µm column and a reverse phase test mix to give you immediate hands-on familiarity with the operation of the Series 4.

As you follow these step-by-step instructions, you will become familiar with the Series 4 pump module controls and the Control Module keyboard. Additionally, you will understand how the parameters are entered to create the Analytical Method (the set of parameters which are used to perform a Chromatographic Analysis) and how the Method may be modified, stored, retrieved. In the course of generating, running, and modifying an untimed isocratic Method, a timed isocratic Method and a simple linear gradient Method, you will learn how to use the Directory and how to use the Hold and Pause keys. If you have an autosampler, there is also a familiarization exercise to introduce chained Methods.

7.1. MATERIALS NEEDED

- Perkin-Elmer High Speed C8 (5 µm) column, P/N 0258-1002, Perkin-Elmer High Speed C18 (5 µm) column, P/N 0258-1001, or a comparable column.

  Our examples were made with a high speed 5 µm C18 column.

- Perkin-Elmer Standard Reverse-Phase Test Mix, P/N 0089-0934. This Test Mix is : 0.2% each: benzene, toluene, ethyl-benzene, isopropyl-benzene, T-butyl-benzene; made up in acetonitrile. Inject 3-5 µl at 250 or 254 nm at a detector setting of 0.32 A_{1cm}. Mobile phase: 60% acetonitrile/water at 2 ml/min.

- Acetonitrile and Water for analyses.

7.2. THE SERIES 4 METHOD

The set of parameters which are used to perform a chromatographic analysis are referred to in this manual as a Method. The Series 4 Method will include solvent composition and times and, if the optional oven is installed, temperature. The Series 4 Method, the sample preparation scheme, the chromatographic conditions, detector settings, and the data processing parameters are all part of the total Analytical Method.

We will concern ourselves here with the Series 4 (pump) Method only.

7.3. UNTIMED ISOCRATIC ANALYSIS

The first Method you will generate and run is an untimed isocratic method. Untimed isocratic analysis are used primarily for methods development and optimization when you simply want the pump to run for an indefinite period of time at a set flow rate and percent compsition as you inject the sample, observe the results, and change the conditions for another try.

7.3.1. Generating an Untimed Isocratic Analysis

Turn on the power to the Series 4 at the Control Module and then at the Pump Module. The Directory Screen Display will appear. The prompt line will say:

POWER FAILURE ENTER PASSWORD

Press the ENTER key to default the password entry. If the Memory protect switch is off and/or no Methods have been previously generated, the screen will clear and the Method Generation Screen Display (see Figure 7-1) will appear. If Methods have been previously stored and the Memory was protected, it would be necessary to press the RESET key and hold it down while pressing the GEN key.

```
METHOD   GEN                                                  TIME      MINS

SECTOR    TIME    FLOW
                  MLS CURVE     A%        B%        C%        D%        CURVE

EQUIL
  1
  2
  3
  4
  5
READY TIME              PMIN        MPa   PMAX        MPa   TEMPERATURE


ENTER METHOD DESCRIPTION ■
```

Figure 7-1 - Method Generation Screen Display Before any Parameters are Entered

When the power is first turned on, the Series 4 goes automatically to the Method Generation mode following entry or default of the password. To enter the Method Generation mode from another mode:

(1) Press the RESET key and hold it down while pressing the GEN key on the Control Module. The prompt line will say:

PRESS METHOD, CHAIN, OR CE

(2) Press the METH key. The prompt:

CLEAR CURRENT METHOD (Y/N)?

will appear.

(3) Type Y. The Screen will clear and the Method Generation Screen Display will appear.

When generating a Method, the user may enter all the parameters from the keyboard or may enter the default values which are permanently stored in the Series 4 memory by pressing the ENTER key.

In this first exercise, we shall enter only the required parameters, and will use the default values for the other parameters. The default values permanently stored in the Series 4 memory are shown in Table 7-1.

TABLE 7-1 - PARAMETER RANGES AND DEFAULT VALUES

PARAMETER	RANGE	DEFAULT VALUE
METHOD DESCRIPTION	40 alpha-numberic characters	None
SOLVENT RESERVOIR IDENTIFICATION	up to 5 alpha-numeric characters	None
EQUIL TIME	0.1 - 999.9 mins.	10 minutes
FLOW - MLS	0.1 - 9.9 ml/min.	2.0 ml/min.
FLOW - CURVE	0.5 - 5.0	0 (zero is a step function)
PERCENT SOLVENT RESERVOIRS A-D	0.0 - 100.0% for each* *Total must not exceed 100%	None (% SOLVENT Must always be entered)
EQUIL TIME	0.0 - 999.9 min.	10 min.
Sector 1-5 TIME	0.0 - 999.9 mins.	0 (zero)
EQUIL FLOW - MLS	0.1 - 9.9 ml/min.	2.0 ml/min.
Sector 1-5 FLOW - MLS	0.1 - 9.9 ml/min.	2.0 ml/min.
Sector 1-5 FLOW - CURVE	0.5 - 5.0	0 (zero is a step function)
SECTOR 1-5 PERCENT SOLVENT RESERVOIRS A-D	0.0 - 100.0% for each* *Total must not exceed 100%	None (% SOLVENT Must always be entered)
READY TIME	0.0 - 999.9 mins.	INFINITE if 0 is entered in Sector 1 TIME (untimed isocratic analysis); 60.0 if a value greater than 0 is entered in Sector 1 TIME (timed isocratic analysis or gradient or flow program analysis).
PMIN	0 - 41.9 MPa*	0 MPa.
PMAX	0.1 - 42 MPa* *PMIN must be always less than PMAX.	42.0 MPa
TEMPERATURE	35°C - 99°C	OFF

The prompt line on the Screen Display will read:

ENTER METHOD DESCRIPTION.

The cursor will be on the top line. Next to METHOD, the system will have automatically entered "GEN".

(4) Type TEST #1 and press the ENTER key to enter the Method Description and to step to the next data entry position.

The prompt line on the Screen Display will read:

ENTER SOLVENT NAME FOR RESERVOIR A.

(5) Type in ACN and press the ENTER key.

The solvent identification for reservoir A which you just typed in will appear in the position over A%. The prompt will read:

ENTER SOLVENT NAME FOR RESERVOIR B.

(6) Type in H2O and press the ENTER key.

The solvent identification for reservoir B will be entered. The prompt will read:

ENTER SOLVENT NAME FOR RESERVOIR C.

(7) Press the ENTER key to default the entry since this reservoir will not be used.

The prompt will read:

ENTER SOLVENT NAME FOR RESERVOIR D.

(8) Press the ENTER key to default the entry since this reservoir will not be used.

In this exercise we are identifying only the 2 reservoirs used in the analysis. A reservoir must be identified in order to use it in a Method. Note that identifying a reservoir is not what controls the actual solvent composition being pumped. This is controlled by the entry under Percent Composition.

If you attempted to default all 4 reservoirs identifications, the Series 4 would return to the first solvent reservoir identification position and the prompt ENTER SOLVENT NAME FOR RESERVOIR A would appear. The Series 4 will not allow you to pass a data entry position in which information has been incorrectly entered.

The cursor will be to the right of EQUIL in the TIME column. For an untimed isocratic analysis, any value between 0.1 and 999.9 minutes may be entered as Equilibration Time. The default value for Equilibration time is which we will use in our analysis 10 minutes.

(9) Press the ENTER key to obtain the default equilibration time of 10 minutes. The system will enter 10 under TIME in the EQUIL Sector, and the cursor will move to the next data entry position which is headed FLOW/MLS. The Flow Rate in ml/min. may be any value between 0.1 and 9.9 ml/min. We shall use the default value of 2 ml/min. in our analysis.

> **Note:** For repetitive runs under the same conditions, the EQUIL TIME can be very short.

(10) Press the ENTER key to obtain the default flow rate of 2 ml/min. The system will leave 2 under FLOW/MLS and the cursor will move to the next data entry position which is headed A%.

(11) Type in 80 and press the ENTER key.

80% will be entered as the percentage of solvent to be taken from Reservoir A.

The Series 4 will automatically compute the percentage of solvent to be taken from the last identified reservoir so that the total of the percentages is equal to 100%. The Series 4 will automatically enter 20 as the percent solvent to be taken from Reservoir B.

> **Note:** If you attempt to enter the last percentage, the numbers you type in will be read by the Series 4 as a TIME entry in the following Sector. If this happens you can clear the entry by using the left arrow key to return to the TIME - Sector 1 data entry position and using the CE (clear entry) key to remove the improper value. The correct time value must be typed in since merely pressing the ENTER key would re-enter the incorrect time value.

The cursor will now be in the TIME column to the right of the "1" in the Sector column. Entering 0 in the TIME data entry position for Sector 1 indicates to the Series 4 that this is to be an untimed isocratic analysis.

(12) Press the ENTER key to obtain the default value of 0 for Sector 1 Time. (Note that the default value for EQUIL TIME is 10 minutes, but the default value for TIME in sectors 1-4 is zero.)

Notice that the Series 4 has automatically entered INF under READY TIME.

Ready Time is the amount of time that the system will remain "ready" and waiting to run without automatically shutting down. Since in the Untimed Isocratic mode, after the initial equilibration time has expired the pump begins to run and runs continuously until it is stopped manually the operator, "ready time" is equal to infinity. Also, since the system remains ready once the equilibration times out, and since the pump is running continuously, the READY/RUN button is disabled in this mode.

When a zero is entered in Sector 1 time, the Series 4 understands this to mean that it is to continue to run under the conditions specified in the EQUIL sector for an infinite time until it is manually stopped by the operator.

The cursor will now be at the PMIN data entry position. Any value for minimum system pressure between 0 and 42 MPa may be entered or the default value of 0 may be entered. A reasonable value for PMIN would be 0.5 - 2.0 MPa. The PMIN value entered, however, must be lower than the value entered for PMAX at the next data entry position.

Note: If the system does not reach PMIN within one minute, the Series 4 will assume that a leak exists or that the reservoir being used has run out of solvent and the pump will automatically shut down. Therefore, it is a good idea to enter a value greater than zero for PMIN rather than defaulting the entry when the system is to run attented.

(13) Press the ENTER key to obtain the default value for PMIN of 0.0 MPA. The system will enter 0.0 under PMIN and the cursor will step to the PMAX data entry position.

Any value for maximum system pressure between 0.1 and 42 MPA may be entered but the default value of 42 MPA is normally used unless the column is fragile.

The value entered for PMAX must be greater than that previously entered for PMIN. If a value is entered for PMAX which is less than or equal to the value entered for PMIN, the Control Module will beep, and the prompt will ask for a new value for PMAX.

If the value for PMAX is ever reached the system will shut down automatically

(14) Press the ENTER to obtain the default value for PMAX of 42 MPA (6000 psi). The system will enter 42 MPA under PMAX. If the oven is connected the cursor will step to the Temperature data entry position.

If the optional column oven is not connected, this parameter will be skipped by the Series 4.

The default value for temperature is ambient. If the column oven is to be used, enter a value between 35°C and 99°C. If the value entered is between 10°C and 35°C, the program will default to OFF, which means that that temperature is not being controlled.

(15) Press the ENTER Key to obtain the default value of ambient tmperature if your Series 4 has the optional column oven connected. OFF will be entered under temperature.

When parameter entries are complete, the Series 4 will automatically leave the Method Generation mode.

The screen display will now look like the one shown in Figure 7-2.

```
METHOD    TEST 1                                                TIME      MINS

SECTOR    TIME    FLOW          ACN        H2O
                  MLS CURVE     A%         B%        C%        D%        CURVE

EQUIL     10.0    2.0           80.0       20.0
  1        0.0
  2
  3
  4
  5
READY TIME   INF      PMIN   0.0   MPa  PMAX   42.0  MPa   TEMPERATURE  OFF


VIAL     INJECT                TOTAL              CURRENT                PRESSURE
         NO.                   TIME               SECTOR      TIME         MPa
                                                  SHTDN        0.0         0.0

■                        FLOW        ACN        H2O

 CURRENT VALUES          0.0         0.0        0.0
```

Figure 7-2 - Screen After Method Generation is Complete. Note that the Current Values Section now appears on the lower portion of the screen.

7.3.2. Storing the Untimed Isocratic Method

(1) Check the screen display to see that the required parameters have been entered correctly.

(2) Press the RESET key and hold it down while pressing the STORE key.

(3) The screen prompt will request:

ENTER METHOD STORAGE NUMBER

(4a) Press the ENTER key to have the system automatically assign the next available storage number to the Method; in this case, #1, or

(4b) Type in number 1 and press the ENTER key. The Method will be stored under this method number. Entering a number which has already been used to store a Method previously will cause the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

to appear.

(5a) Answering N will exit from the store program. Go back to step (2) and repeat the storage process, assigning a different storage number or using the ENTER Key to assign the next available number.

(5b) Answering Y will replace the Method stored under that number with the Method presently shown on the screen display.

Note: An Untimed Isocratic Method cannot be used in a Chain. Because of this limitation, an Untimed Isocratic Method cannot replace in storage a Timed Isocratic Method which has been used in a Chain.

7.3.3. Recalling the Method from Storage

The Method displayed on the screen must be in Shutdown in order to be replaced by a Method from storage.

(1) Recall the stored Method to the screen by pressing the RESET key and holding it down while pressing the METH key. The prompt will ask for the Method number.

(2) Type in the number of the Method to be called to the screen from storage and press the ENTER key.

7.3.4. Degassing (Sparging) The Solvents

(1) Rotate the pressure regulator knob (see Figure 7-3) counterclockwise 3 or 4 turns to close the valve.

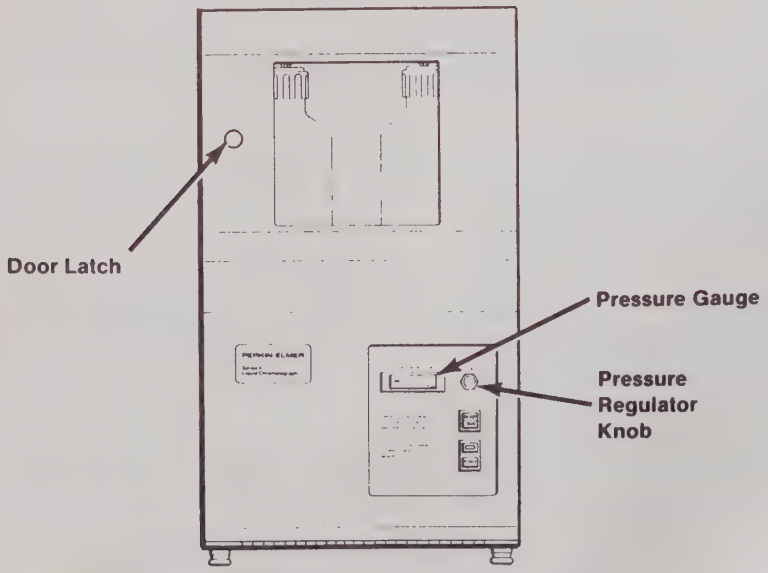


Figure 7-3 - Pressure Regulator Knob Location

(2) Remove the reservoirs from the solvent chamber. Notice that the solvent lines are labeled A, B, C and D. Fill Reservoir A with Acetonitrile (ACN) and Reservoir B with water (H_2O). Cap the lines for C and D. The helium input line cap is P/N 0254-2416. The vent line cap is P/N 0254-2415.

(3) Replace the caps on the reservoirs, making sure that the correct solvent line leads from each reservoir (the lines are tagged A, B, C and D). Turn the reservoirs rather than the caps to avoid twisting the lines. Place the reservoirs in the solvent chamber.

(4) With the door to the solvent chamber OPEN, rotate the presssure regulator knob clockwise to start the helium.

(5) Sparge (bubble) the solvents for approximately 5 minutes.

7.3.5. Pressurizing The Solvents

(1) Close the door to the solvent chamber and turn the knob to latch it. The reservoirs will then automatically pressurize. The bubbles will slow and eventually stop forming.

> **Warning:** Whenever the door to the solvent chamber is opened, the solvent reservoirs depressurize automatically. This is a safety feature. Do not attempt to defeat the door interlock.

(2) Adjust the pressure by rotating the presssure regulator knob (see Figure 7-3) clockwise until the pressure gauge reads 7 to 9 psi.

> **Important:** Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

7.3.6. Priming The Pump And Purging The System

Note: A reservoir which has not been identified in the Method cannot be used to purge.

(1) Open the cover to the pump compartment as shown in Figure 7-4. (The cover to the pump compartment is hinged on the bottom.)

(2) Connect the priming syringe to the flush port (see Figure 7-4).

(3) On the Control Module press the blue RESET key and hold it down while you press the PURGE key. The prompt line will say:

RESERVOIR?

(4) Type in the letter A. The prompt line on the CRT Screen Display will say:

PURGE FLOW?

Note: If you type in anything other than the letters A, B, C or D, the prompt:

RESERVOIR?

will re-appear. Type in the correct letter and press the ENTER key.

(5) To get a flow rate of 0 (zero), press the ENTER key*.

(6) Go back to the Pump Module. Open the drain valve by turning the handle counterclockwise (see Figure 7-4 for the location of the drain valve).

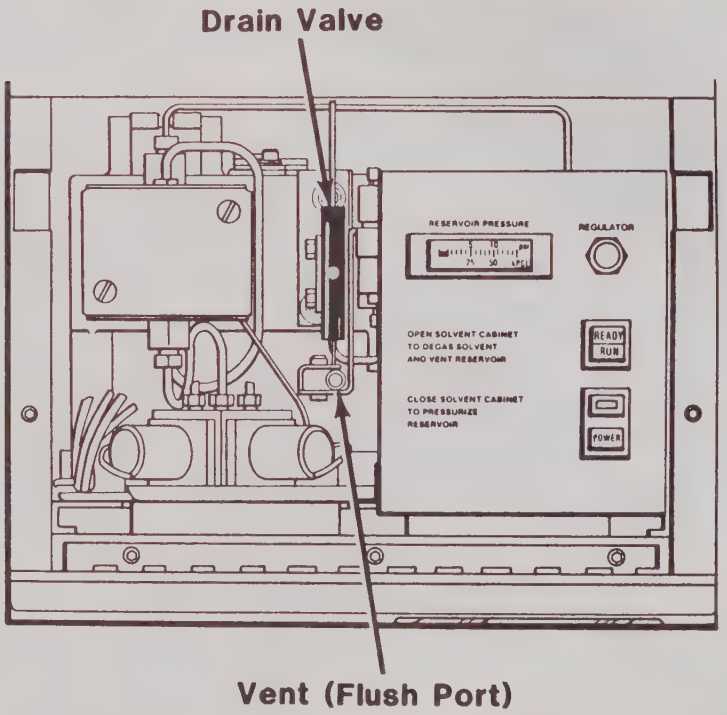


Figure 7-4 - Drain Valve and Flush Port Locations

(7) Collect about 20 milliliters of solvent in the syringe or continue until air bubbles have stopped coming through.

(8) Stop the flow into the syringe by turning the drain valve handle clockwise. Make sure that the valve is fully closed before removing the priming syringe.

*Note: A purge flow rate is used when the column is to be purged. Pick a pump flow rate appropriate to the backpressure of the column.

(9) Press the blue RESET key and hold it down while you press the STOP key. This will exit from the PURGE mode.

(10) Remove the priming syringe and dispose of the solvent in an appropriate waste container.

(11) Reconnect the priming syringe to the flush port.

(12) On the Control Module press the blue RESET key and hold it down while you press the PURGE key. The prompt line on the Screen Display will say:

RESERVOIR?

(13) Type in the letter B and press the ENTER key. The prompt line on the Screen Display will say:

PURGE FLOW?

(14) Press the ENTER key.

(15) Open the drain valve on the Pump Module by turning the handle counterclockwise.

(16) Collect about 20 milliliters of solvent in the syringe or continue until air bubbles have stopped coming through.

(17) Stop the flow into the syringe by turning the drain valve handle clockwise.

(18) Press the blue RESET key and hold it down while you press the STOP key. This exits from the Purge Mode.

(19) Remove the syringe and dispose of the solvent in the waste container.

7.3.7. Running The Untimed Isocratic Method

(1) Press the RESET key and hold it down while pressing the START key. This will start the pump. following the expiration of the equilibration time, the READY light on the Pump Module will light. This light will remain on until the pump is manually stopped by the operator.

(2) Inject the sample. Watch the Current Values.

(3) Watch the chromatogram. Your chromatogram will probably look similar to that shown in Figure 7-5.

(4) Push the RESET key and hold it down while pressing the STOP key.

Figure 7-5 - Chromatogram of Run Using Method of Figure 7-2 (Test #1)

7.3.8. Modifying The Untimed Isocratic Method

In this mode the Method may be modified at any time by stepping to the EQUIL sector.

(1) Press the RESET key and hold it down while pressing the blue EQUIL key (do not confuse the blue EQUIL key with the white TEQ parameter entry key). The program will step immediately to the EQUIL sector.

Note: If you attempt to modify a Method while the program is in READY or RUN, the Control Module will beep and the prompt will say:

MODIFY METHOD IN EQUIL OR SHUTDOWN ONLY

Let's change the %A to 60% and change the minimum presure from 0 to 0.5 MPa.

(1) Press the RESET key and hold it down while pressing the MOD key. The system will enter MODIFY next to "Test #1" as a "flag" to the user that the system is in the "Modify" mode. Press ENTER to step through or RESET and PARAMETER key to go directly to parameter to be modified.

Note: Once you have entered the "Modify" mode, you can only escape by pushing the CE key before making any modifications to the Method. Otherwise you must step through all the parameters to exit before attempting to run or store the Method. This is easily done by rapidly pressing the ENTER key or using the REPEAT key. If nothing happens when you try to store or run a Method, check to see whether the Method is in the "Modify" mode.

(2) Press the ENTER key once to default the Method Description. The terminal will beep and the prompt:

DUPLICATE METHOD NAME. PRESS Y TO CHANGE OR ENTER TO CONTINUE

will appear.

(3) Press the ENTER key 3 times. The cursor should be at the A% data entry position.

If you have inadvertently passed the data entry position for A%, use the SHIFT key in conjunction with the left arrow key to return to the EQUIL line. Then use the ENTER key to step across to the A% data entry position.

(4) Type 60 and press the ENTER key. 60% will be entered as the % solvent A and 40% will automatically be entered as the percentage from Reservoir B.

(5) Press the ENTER key once.

The cursor should now be at the PMIN data entry position.

Note: If you inadvertently pass the PMIN data entry position, again, use the shift and the left arrow keys to return to PMIN position.

(6) Type in 0.5 and press the ENTER key. The minimum pressure will now be 0.5 MPa.

(7) Press the ENTER key once if the optional column oven is not connected and twice is the oven is connected. This will terminate the MODIFY routine.

The Screen Display will now look like the one shown in Figure 7-6.

(8) When modification is complete, press the RESET key and hold it down while pressing the START key. When the equilibration times out, the Ready light will illuminate.

```
METHOD    TEST 1                                                  TIME      MINS

SECTOR    TIME    FLOW        ACN       H2O
                  MLS CURVE   A%        B%        C%        D%        CURVE

EQUIL     10.0    2.0         60.0      40.0
  1        0.0
  2
  3
  4
  5
READY TIME   INF       PMIN  0.5   MPa   PMAX   42.0 MPa  TEMPERATURE  OFF


VIAL     INJECT              TOTAL              CURRENT              PRESSURE
         NO.                 TIME          SECTOR      TIME          MPa
                                           SHTDN        0.0          0.0

■                    FLOW        ACN       H2O

CURRENT VALUES       0.0         0.0       0.0
```

Figure 7-6 - Untimed Isocratic Method After Modifications

Notice that the CURRENT VALUES Section of the Screen Display shows the actual state of the pump module from the previous run.

(9) Inject the sample.

(10) Watch the chromatogram. Your chromatogram should look similar to that shown in Figure 7-7. Notice the decrease in retention time from the previous run.

In the untimed isocratic mode, you can continue in this manner, modifying the parameters as necesssary, until you get a satisfactory analysis.

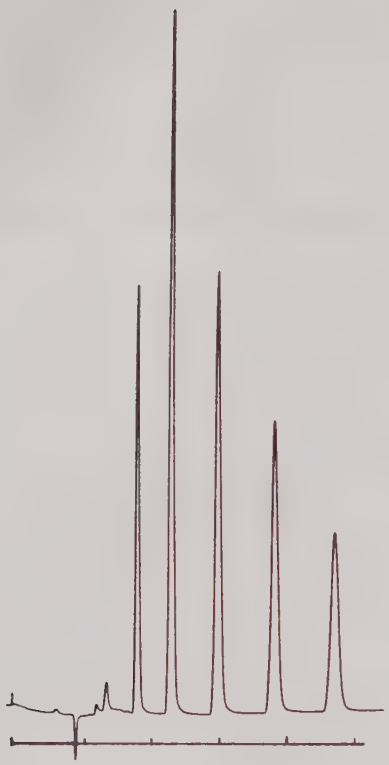


Figure 7-7 - Chromatogram of Run Using Untimed Isocratic Method after Modifications

7.3.9. Stopping The Pump

As mentioned earlier, the Series 4 will continue to run in the untimed isocratic mode until it is stopped by the operator or, if a minimum pressure has been entered, the system drops below the minimum pressure when the reservoir runs out of solvent or if the maximum pressure entered is reached.

(1) Press the RESET key and hold it down while pressing the STOP key. This will stop the pump and abort any analysis in progress.

In this familiarization exercise we have not used the column oven. However, it is important to remember, that when a heated oven is used with an untimed isocratic run, there is no automatic cool-down sequence to protect the column. The operator must modify the Method without stopping the pumps to turn off the oven and then must allow the solvent to continue to flow for a sufficient time to cool down the column.

To provide a cool-down sequence to protect the column after running in the untimed isocratic mode:

(1) Press the RESET key and hold it down while pressing the EQUIL key. This will step to the equilibration sector.

CAUTION: DO NOT STEP TO THE SHUTDOWN SECTOR TO MODIFY THE TEMPERATURE

(2) Press the RESET key and hold it down while pressing the MODIFY key.

(3) Press the TEMP parameter key to step directly to the temperature parameter.

(4) Type 10* and press the ENTER key. This will enter 10°C, which will turn the oven OFF.

(5) Allow the solvent to flow for a sufficient period of time to cool the column or remove the column to protect it.

7.4. TIMED ISOCRATIC ANALYSIS

Once a method has been developed and the optimum parameters to produce a satisfactory analysis for that sample type are known, the user can store the parameters, or Method for that analysis so that it may be recalled at will or used in a chain, along with gradient methods if desired, for unattended isocratic analyses. The internal clock on the Series 4 is used to control analysis time.

As we saw earlier, there are two ways to create a Method. The first is to generate a new Method; the second is to modify an existing Method, changing specific parameters. We will modify the untimed isocratic analysis we just generated to make it a Timed Isocratic Analysis Method.

7.4.1. Modifying An Existing Method

If the Untimed Isocratic Method is not currently displayed, recall it to the screen.

(1) Press the RESET key and hold it down while pressing the MOD key. The system will automatically write "MODIFY" next to METHOD. The prompt

SELECT SECTOR OR PRESS ENTER KEY

will appear.

(2) Press the ENTER key to step through the parameters. The prompt will read:

ENTER METHOD DESCRIPTION Test #1

and the cursor will be on the "T" of "Test".

(3) Type "TEST #2" and press the ENTER key. The system will enter this as the Method Description and step to the next parameter entry position.

(4) Press the ENTER key 5 times. This will leave the solvent reservoir identifications and all the parameters entered in the EQUIL sector of the Method unchanged. The cursor should be in Sector 1 under TIME. If you have accidentally passed this data entry position, use the left arrow key to return to it.

*Any value between 10 and 34 may be used.

A Timed Isocratic Analysis uses the EQUIL Sector for Equilibration and Sector 1 for the analysis. The only entry in Sector 1 which will differ from the EQUIL Sector entries is the TIME entry.

(5) Type 10 and press the ENTER key. Check the Screen Display to make sure that 10 is shown under TIME in Sector 1.

The cursor will step to the Sector 1 FLOW/MLS entry position.

(6) Type 2 and press the ENTER key.

The cursor will step to the Sector 1 A% position.

(7) Type 60 and press the ENTER key.

The Series 4 will automatically compute the percentage of solvent to be taken from the last identified reservoir (reservior B in this case) and will enter 40 in the Sector 1 B% position.

(8) Press the ENTER key. This will enter 0 (zero) in the Sector 2 TIME entry position.

(9) Press the ENTER key a second time. This will enter a default value of 60 under READY TIME.

Note: When one or more Time Sectors are used, the default value for READY TIME is 60.0 rather than 999.9.

(10) Press the ENTER key twice more if the column oven is not installed, and three more times if the oven is installed. This will default the entries for PMIN, PMAX and TEMPERATURE and will terminate the Modify Mode.

The terminal will beep and the prompt will read:

REMEMBER TO STORE MODIFIED METHOD.

7.4.2. Storing The Timed Isocratic Method:

(1) Press the RESET key and hold it down while pressing the STORE key.

(2) The screen prompt will request:

ENTER METHOD STORAGE NUMBER

(3) Press the ENTER key to have the Method stored under the next available storage number (in this case 2) and press the ENTER key. You could also type in 2 and press the ENTER key.

```
METHOD     TEST 2                                              TIME       MINS

SECTOR     TIME    FLOW          ACN        H2O
                   MLS CURVE     A%         B%        C%        D%        CURVE

EQUIL      10.0    2.0           60.0       40.0
  1        10.0    2.0           60.0       40.0
  2         0.0
  3
  4
  5
READY TIME  60.0        PMIN  0.5   MPa   PMAX  42.0  MPa  TEMPERATURE OFF


VIAL     INJECT                  TOTAL          CURRENT              PRESSURE
         NO.                     TIME       SECTOR      TIME          MPa
                                            SHTDN        0.0          0.0

                    FLOW         ACN        H2O

CURRENT VALUES       0.0         0.0        0.0
```

Figure 7-8 - Timed Isocratic Method

(4) If you had typed in a number which had already been used, the prompt:

OVERWRITE EXISTING METHOD (Y/N)?

would appear.

(5) Since this Method is to be stored separately, you would type N and press the ENTER key. Thie would exit from the storage mode.

Then you would re-enter the Storage mode and begin again.

Check the Method Description line to confirm the storage number for the Method. In this case, the system should assign #2. The top line of the screen display should say:

METHOD 2 STORED TEST #2

7.4.3. Running The Timed Isocratic Method:

(1) Press the RESET key and hold it down while pressing the START key. This will start the pump. The system will equilibrate for the 10 minutes shown under EQUIL TIME. When the equilibration time has passed, the system will become "Ready" and the READY Light on the Pump Module will light.

(2) Inject the sample.

(3) Immediately press the RUN button on the Pump Module.

(4) Watch the chromatogram.

The pump will run for the time entered in Sector 1 (15 minutes) and then the system will automatically go back to the equilibration sector and the system will equilibrate for the 10 minutes shown under EQUIL TIME. When Equilibration times out, the READY Light will light on the Pump Module. If the RUN button on the Pump Module is pressed again, the pump will run for the time entered in Sector 1, automatically return to the equilibration sector and, when equilibration times out, the Series 4 will become "Ready" again.

If the RUN button on the Pump Module is NOT pressed, the system will remain "Ready" and the pump will run for the time entered under READY TIME. Then the Series 4 will automatically shut down.

If an oven is connected to the Series 4 and is heated, when the Ready Time runs out, the Series 4 will enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. This cool-down sequence protects the column. When it is complete the Series 4 will shut down.

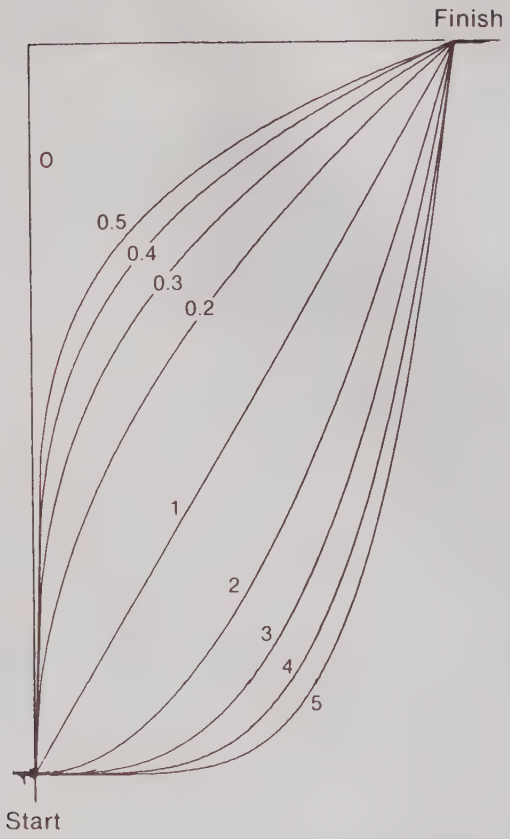
CAUTION: The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

(5) Push the RESET key and hold it down while pressing the STOP key to stop the pump.

7.5. GRADIENT ANALYSIS

If a change in the elution strength of the mobile phase with time is necessary to obtain a satisfactory chromatogram, a gradient program is used. In a simple linear gradient program (curve 1) the gradient is produced by the gradual increase in percentage of the stronger solvent and simultaneous reduction of the weaker solvent while a constant flow rate is mantained*. Concave gradients (curves 2, 3, 4 and 5) are used to maintain early resolution and sweep off later components. Convex gradients (curves 0.2, 0.3, 0.4 and 0.5) are used to speed up the early peaks and maintain resolution of the later peaks. The linear and concave gradients are the most useful in LC. Figure 7-9 shows the gradient curves available on the Series 4.

*Note: It is possible to combine a gradient program with a flow program on the Series 4. This is discussed in the Operation Section.



Note: Because fractional entries cannot be made on the Series 4, 0.2, 0.3, 0.4, and 0.5 are used by convention to indicate convex gradients.

Figure 7-9 - Gradient Curves Available on the Series 4.

For difficult separations, the Series 4 allows four solvents to be employed in a gradient to combine multi-solvent technology and gradient elution as in cases when samples have widely differing polarity or when a system modifier is necessary for pH control, ion-pairing or ion suppression.

Once a Method has been developed and the optimum parameters for a satisfactory analysis are known, the user can store the gradient analysis Method for later use or for inclusion in a Method Chain for unattended analyses.

For Gradient analysis, the Setup, Sparing, Purging and Solvent Pressurization procedures are the same as for ioscratic analyses.

Figure 7-10 shows a linear gradient shown as a plot of % strong solvent against time. The time base is shown divided into an EQUIL sector and 3 time sectors followed again by the EQUIL sector.

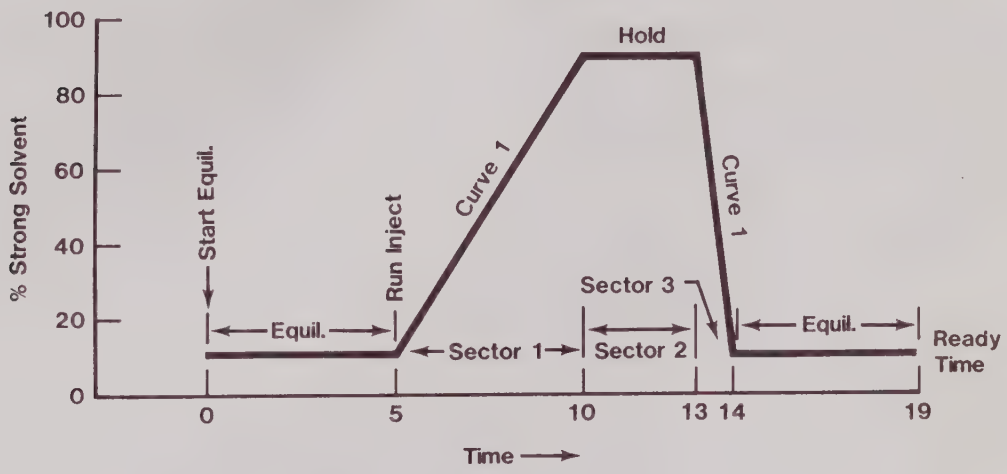


Figure 7-10 - Linear Gradient Shown as % of Strong Solvent Against Time

7.5.1. Generating A Gradient Method

To generate the Gradient Method shown in Figure 7-10, we want the system to equilibrate for 5 minutes under starting conditions. In Sector 1, the linear gradient is run: the % strong solvent changes from 10% to 90% linearly over a 10 minute time period. In Sector 2, the system runs isocratically for 3 minutes to make sure that all highly-retained materials are swept off the column. In Sector 3, the system once again runs a linear gradient--this time a ramp back to the initial conditions. The system goes from 90% to 10% strong solvent over a 1 minute time period. At the end of Sector 3, the Series 4 returns to the EQUIL Sector, and after the 5 minutes of equilibration time have passed, the READY light comes on.

Let's enter these parameters.

(1) Press the RESET key and hold it down while pressing the GEN key.

(2) The prompt will say:

PRESS METHOD, CHAIN, or CE

(3) Press the METH key. The prompt will say:

CLEAR CURRENT METHOD (Y/N)?

(4) Press Y. The Method Generation Screen Display will appear. The prompt will say:

ENTER METHOD DESCRIPTION.

(5) Type in "TEST #3" and press the ENTER key. The prompt will say:

ENTER SOLVENT NAME FOR RESERVOIR A.

(6) Type in ACN and press the ENTER key.

The prompt will say:

ENTER NAME FOR RESERVOIR B.

(7) Type in H_2O and press the ENTER key.

The prompt will say:

ENTER SOLVENT NAME FOR RESERVOIR C.

(8) Press the ENTER key. Reservoir C will not be used.

The prompt will say:

ENTER SOLVENT NAME FOR RESERVOIR D.

(9) Press the ENTER key. Reservoir D will not be used.

The cursor will step to the EQUIL TIME data entry position.

EQUIL SECTOR ENTRIES

(10) Type 5 and press the ENTER key.

The cursor will step to the equilibration sector FLOW/MLS entry position.

(11) Press the ENTER key. The system will enter the default value of 2.

The cursor will step to the % SOLVENT data entry position for Reservoir A.

(12) Type in 10 and press the ENTER key.

Since only 2 reservoirs were identified, the system will enter 90 as the % SOLVENT for Reservoir B automatically and the cursor will step directly to the Sector 1 TIME data entry position.

SECTOR 1 ENTRIES

(13) Type 5 in the Sector 1 TIME parameter and press the ENTER key.

The cursor will step to the Sector 1 FLOW/MLS data entry position.

(14) Press the ENTER key. The system will enter the default value of 2.

> **Note:** If a different value for FLOW/MLS were entered in Sector 1 than was entered in the EQUIL sector, the cursor would step to the FLOW/CURVE data entry position. The flow curves available on the Series 4, like gradient curves, are concave, linear and convex.
>
> However, the limitation on the Series 4 is that only 2 curves may be programmed into one time sector; one flow curve and one solvent curve or two solvent curves may be programmed simultaneously. If more than 2 curves are entered in a time sector, the prompt will read:
>
> TOO MANY CURVES
>
> and the cursor will return to the data entry position where the correction must be made. Asterisks will appear next to each curve entry. See the Operator Aids Section - How the Series 4 Counts Curves.

If the flow rate of 2 ml/min. of step 11 is entered correctly, the cursor will not stop at the FLOW/CURVE data entry position, but will step directly to the % SOLVENT for Reservoir A data entry position*.

(15) Type in 90 and press the ENTER key.

Since only 2 reservoirs were identified, the system will enter 10 as the percent solvent to be taken from Reservoir B.

The cursor will step to the CURVE data entry position. Permissible entries in this data entry position are 1 (for a linear gradient); 2, 3, 4 or 5 (for concave gradients); and 0.2, 0.3, 0.4 and 0.5 (for convex gradients).

Refer to Figure 7-9 for a graphic representation of the curves.

(16) Type in 1 (since this is a linear gradient) and press the ENTER key.

The cursor will step to the Sector 2 TIME data entry position.

SECTOR 2 ENTRIES

(17) Type in 3 for the Sector 2 TIME parameter and press the ENTER key.

The cursor will step to the Sector 2 FLOW/MLS data entry position.

*If the flow rate in Sector 1 were different, this would be a flow curve. Flow curves are discussed in the Operation Section of this manual.

(18) Press the ENTER key. The system will enter the default value of 2.

Since this flow is the same as that entered in Sector 1, the cursor will not stop at the FLOW/CURVE data entry position but will step directly to the % SOLVENT for Reservoir A data entry position.

(19) Type in 90 and press the ENTER key. Note that this entry, and the entry for Reservoir B will be the same as the final conditions at the end of Sector 1.

The system will calculate the % solvent for Reservoir B and enter 10 automatically.

The cursor will step directly to the Sector 3 TIME data entry position.

SECTOR 3 ENTRIES

(20) Type in 1 for the Sector 3 TIME parameter and press the ENTER key.

The cursor will step to the FLOW/MLS position.

(21) Press the ENTER key to obtain the default value of 2 ml/min..

The cursor will step to the % SOLVENT for Reservoir A position.

(22) Type in 10 and press the ENTER key.

The system will automatically calculate the % solvent for reservoir B and enter 90. the cursor will step to the CURVE data entry position.

(23) Type in 1 (since this is, once again, a linear gradient) and press the ENTER key. The cursor will step to the Sector 4 TIME data entry position.

SECTOR 4 ENTRY - END OF SECTOR ENTRIES

(24) Press the ENTER key to obtain default value of 0.

A zero is entered in the TIME entry position in Sector 4 to indicate to the Series 4 that Sectors 4 and 5 will not be used in this Method. The cursor will move to the READY TIME entry position

(25) Type 45 and press the ENTER key to obtain a READY TIME of 45 minutes.

The System will step to the PMIN data entry position.

(26) Type in 0.5 and press the ENTER key.

The cursor will step to the PMAX position.

(27) Press the ENTER key to obtain the default value of 42 MPa. If the column oven is not connected, the Method Generation is now complete. If the column oven is connected to the Series 4, the cursor will step to the TEMPERATURE data entry position.

(28) Press the ENTER key since temperature control will not be used, OFF will be entered by the System. The Method Generation is now complete.

If the temperature were to be used, it is important to remember that when a timed run is programmed with a heated oven, after the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump duirng the cool-down sequence.

The Screen Display should look like the one shown in Figure 7-11.

```
METHOD      TEST 3                                          TIME 14.0 MINS

SECTOR    TIME    FLOW          ACN       H2O
                  MLS CURVE     A%        B%        C%        D%        CURVE

EQUIL      5.0    2.0           10.0      90.0
  1        5.0    2.0           90.0      10.0                          1
  2        3.0    2.0           90.0      10.0
  3        1.0    2.0           10.0      90.0                          1
  4        0.0
  5
READY TIME   14.0   PMIN  0.5 MPa   PMAX  42.0 MPa   TEMPERATURE  OFF


VIAL      INJECT                TOTAL              CURRENT              PRESSURE
          NO.                   TIME          SECTOR       TIME          MPa
                                              SHTDN        0.0           0.0

■                   FLOW        ACN       H2O

 CURRENT VALUES      0.0         0.0       0.0
```

Figure 7-11 - Linear Gradient Method Screen Display

7.5.2. Storing The Gradient Method

(1) Check the screen display to see that the required parameters have been entered correctly.

(2) Press the RESET key and hold it down while pressing the STORE key.

(3) The screen prompt will request:

ENTER METHOD STORAGE NUMBER?

(4) Press the ENTER key to assign the next available storage number to the method or type in a number which has not been used to store another Method and press the ENTER key.

The Method description line will show the number under which the Method was stored. In this case it should be #3.

7.5.3. Running The Gradient Analysis

The Method to be run must be displayed on the Screen. If it is not, recall it from memory or generate it, following steps outlined above.

(1) Press the RESET key and hold it down while pressing the START key.

The pump will start and the system will equilibrate for 10 minutes. When the equilibration time has passed, the system will become "Ready" and the READY Light on the Pump Module will light. The pump will continue to run, delivering the mixture defined in the EQUIL Sector, for the time entered in the READY TIME parameter unless the RUN button is pressed.

(2) Inject the Sample.

(3) Immediately press the RUN button on the Pump Module.

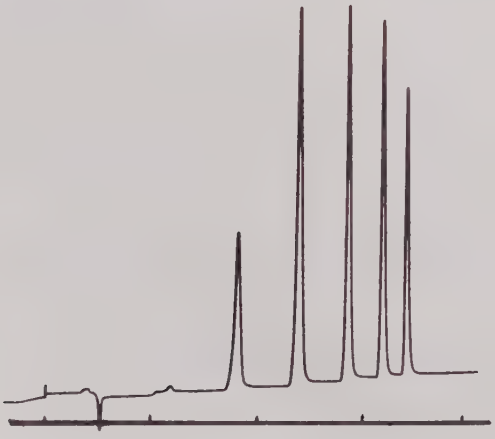
(4) Watch the Chromatogram. It should look similar to that shown in Figure 7-12.

The gradient analysis could be improved by modifying the Method so that the strong solvent changes from 40% to 85% over a 5 minute time period with a 3 minute hold at 85%. If you make this modification, your chromatogram should look similar to the one shown in Figure 7-13.

Starting with Sector 1, the system will step through the sectors and then will return to the equilibration sector. When the equilibration times out, the READY light illuminates on the Pump Module.

If the RUN button on the Pump Module is pressed again, the program will step through the sectors, and automatically return to the equilibration sector. When equilibration times out again, the Series 4 becomes "Ready" again.

Figure 7-12 - Chromatogram of Run Using Linear Gradient Method (see Figure 7-11)



This chromatogram shows how Test 3 could be improved by changing the strong solvent from 40% to 85% over a 5 minute period with a 3 minute hold at 85%.

Figure 7-13 - Improving Test 3 by Changing the Gradient

If the RUN button on the Pump Module is not pressed, the system will remain "Ready" and the pump will run for the READY TIME entered and then the Series 4 will shut down.

If the oven is connected to the Series 4 and is heated, the Series 4 will enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. This cool-down sequence protects the column. When it is complete, the Series 4 will shut down.

7.5.4. Using The Hold And Pause Keys:

The HOLD key may be used to hold the program in the current sector of a Gradient analysis. The clocks stop when this key is used but the pump continues to run. The HOLD key, in effect, adds time to the current sector.

The PAUSE key may be used to temporarily pause the entire system. When the system is restarted, the program will resume where it paused.

7.5.4.1. USING THE HOLD KEY

We will rerun the Gradient Analysis (Test #3) to see how the HOLD and PAUSE keys affect the chromatogram.

(1) Wait for the READY LIGHT On the Pump Module to light again.

(2) Inject the sample.

(3) Press the RUN button on the Pump Module.

(4) Watch the CURRENT VALUES section of the Screen Display. When 10 minutes have passed, you will be in Sector 1 (CURRENT SECTOR).

(5) Watch the ELASPED SECTOR time value.

(6) When 2 minutes have passed, press the RESET key and hold it down while pressing the HOLD key.

HOLD will appear next to the Sector number under the CURRENT SECTOR heading. The Series 4 clocks will stop. The pump will continue to run.

(7) When 2 minutes have passed, press the RESET key and hold it down while pressing the HOLD key again. This will restart the programmed timing.

You have effectively added 2 minutes to the Sector 1 Time.

7.5.4.2. USING THE PAUSE KEY

(1) Watch the CURRENT VALUES section of the Screen Display. When 22 minutes have passed, you will be in Sector 3 (CURRENT SECTOR). Watch the ELAPSED SECTOR time value.

(2) When 1 minute has passed, press the RESET key and hold it down while pressing the PAUSE key. The pump and the Series 4 clocks stop. Both the Sector and Total Elapsed Time counters will be paused. PAUSE will appear next to the Sector number under the CURRENT SECTOR heading. Wait a minute or so.

(3) Press the RESET key and hold it down while pressing the PAUSE key again. The system will restart and the program will resume where it paused.

(4) Watch the chromatogram. Notice that the HOLD key has the effect of adding additional time to the sector in which it is used, while the PAUSE key pauses the entire system and does not alter the programmed conditions.

7.5.5. Stopping The Gradient Analysis

Press the RESET key and hold it down while pressing the STOP key. Alternately the analysis may be stopped by stepping to the EQUIL Sector.

7.6. OBTAINING A DIRECTORY OF MEMORY CONTENTS

A directory of the contents of the Series 4 memory may be obtained at any time* by the user.

(1) Press the RESET key and hold it down while pressing the DIR key.

(2) The Directory Screen display will replace the Method currently shown on the screen. An example of the Screen Display is shown in Figure 7-14. You will see that the System has stored three Methods: Test #1, Test #2 and Test #3.

```
                    DIRECTORY

METHOD NUMBER                    METHOD DESCRIPTION

      1                          TEST 1
      2                          TEST 2
      3                          TEST 3


POWER FAILURE ENTER PASSWORD ■
```

Figure 7-14 - Directory Screen Display

*If you are in the process of generating, modifying or running a Method and you wish to see the Directory, you must finish stepping through the Generate or Modify modes and you should store the Method before calling the Directory to the screen.

(3) To return to the Method Screen Display, press the RESET key and hold it down while pressing the METH key. The prompt will read

CLEAR CURRENT METHOD (Y/N)?

(4) Press N to return to the Method you were working on before you called the directory.

7.7. CHAINING METHODS TOGETHER FOR UNATTENDED ANALYSES

If you have an autosampler, up to 9 methods may be chained together in any order and for any number of times to analyze up to 100 samples with up to 9 injections of each sample.

To connect the Autosampler to the Series 4, see the Installation Section. The Setup, Sparging, Purging and Solvent Pressurization procedures are the same as those given earlier for untimed isocratic analysis.

7.7.1. Generating A Chain

Let's create a simple chain using Methods #2 and #3 which have already been stored in the Series 4 memory. Method #1 cannot be used in a chain since it is an untimed isocratic analysis.

(1) Press the RESET key and hold it down while pressing the GEN key. The prompt will say:

PRESS METHOD, CHAIN, OR CE

(2) Press the CHAIN key.

If no chain as previously been generated, the screen will clear and the CHAIN Screen Display will appear. An example of the screen display is shown in Figure 7-15.

```
NO. OF          VIAL          NO. OF    METHOD      METHOD DESCRIPTION
SAMPLES          NO.          INJECT
                F    L
```

```
GEN>PRESS RESET/STORE TO EXIT CHAIN GENERATION ■
```

Figure 7-15 - CHAIN Screen Page

(4) The first entry required is the number of samples to be analyzed with the first Method in the Chain.

Type 6 and press the ENTER key.

The system will automatically enter the first and last vial numbers in the second and third columns of the Chain Screen Display.

> **Note:** The Autosampler should be loaded according to the vial numbers listed in this display.

(5) The second entry required is the number of injections to be made of each sample.

Type 1 and press the ENTER key.

(6) The third entry required is the number of the Method to be used to analyze the first 6 samples.

Type 2 and press the ENTER key.

The system will automatically enter the Method Description and the system will step to the next line.

> **Note:** The Method number to be entered is the number the Method is stored under in Series 4 memory. If a Method number is entered which has not been used to store a Method in memory the following prompt will appear:
>
> METHOD DOES NOT EXIST, CE
>
> Press the CE (CLEAR ENTRY) key and then re-enter the correct Method number.

(7) The first entry on the second line required is the number of samples to be analyzed with the second Method in the Chain.

Type 4 and press the ENTER key.

The system will automatically enter the first and last vial numbers to be used in the second and third columns of the Chain Screen Display. The Autosampler should be loaded accordingly.

(8) The second entry on the second line required is the number of injections to be made of each sample to be analyzed with the second Method.

Type 1 and press the ENTER key.

(9) The third entry on the second line required is the number of the Method to be used to analyze the four samples contained in vials 7 through 10.

Type 3 and press the ENTER key.

The system will automatically enter the Method Description and the system will step to the next line.

This entry procedure is continued until all the samples (up to 100 with the ISS-100 Autosampler) have been specified.

7.7.2. Storing A Chain

We shall only use 10 samples in our chain. To end the Chain Generation, press the RESET key and hold it down while pressing the STORE key.

PRESS RESET/METH OR RESET/DIR

will appear on the bottom of the Screen Display. The Screen Display will look similar to that shown in Figure 7-16.

```
NO. OF        VIAL      NO. OF    METHOD    METHOD DESCRIPTION
SAMPLES       NO.       INJECT
              F    L

    6         1    6        1         2     TEST 2
    4         7   10        1         3     TEST 3


GEN>PRESS RESET/STORE TO EXIT CHAIN GENERATION ■
```

Figure 7-16 - Sample CHAIN Screen Display after entries have been made

Load the Autosampler according to the Displayed Chain Method.

7.7.3. Running A Chain

7.7.3.1. STARTING THE FIRST METHOD

To run the Chain, the first Method in the Chain must be displayed on the screen and must be running in the EQUIL or READY time sectors.

(1) Press the RESET key and hold it down while pressing the METH key.

The prompt will say:

ENTER METHOD NUMBER

(2) Type 2 (the storage number of the first Method in this Chain) and press the ENTER key. The screen will clear and the Method Screen Display for the Method #2 will appear.

(3) Press the RESET key and hold it down while pressing the START key. The pump will start and the system will go to the EQUIL sector.

7.7.3.2. EXECUTING THE CHAIN

(4) Press the RESET key and hold it down while pressing the EXEC key. This will implement the Chain process. It does not start the run. You must press the RUN button to begin the first analysis. Normally, the Autosampler will provide the RUN signal for subsequent analyses in the Chain.

> **Note:** Unless the Series 4 is connected so that it receives an external RUN signal it will be necessary to press the RUN button each time the system becomes "Ready".

Notice that in the Current Status section of the Screen Display the Vial Number and Injection Numbrs will be filled in by the system and "Chain Running" will appear next to CURRENT SECTOR.

7.7.4. Leaving The Chain

7.7.4.1. EXITING FROM THE CHAIN

To exit from a CHAIN, press the RESET key and hold it down while pressing the EXIT key. This exists from the Chain, but will not stop any analysis in progress.

> **Important:** It is not possible to exit from a Chain and re-enter it subsequently at the exit point. Either the entire Chain must be rerun from the beginning or the Chain must be modified so that the first Method to be run is the first Method in the Chain. If a Chain is to be stopped temporarily, use the PAUSE key rather than the EXIT key to pause the system.

7.7.4.2. STOPPING THE METHOD

To stop an analysis in progress, press the RESET key and hold it down while pressing the STOP key.

The preceding exercises should have given you an overall picture of how easily methods are generated and run with the Series 4. They were not meant to answer all your questions. For more complete instructions on the function of the various controls, read the Controls and Indicators Section. For complete operation instructions, turn to the Operation Section.

8 SERVICE AND MAINTENANCE

Section		Page
8.1	Service	8-1
8.2.	Maintenance	8-1
8.2.1	Electrical Hazards	8-1
8.2.2	Mechanical Hazards	8-1
8.2.3	Routine Checks	8-1
8.2.4	Periodic Checks	8-1
8.2.5	Solvent Input Filters	8-1
8.2.6	Solvent Spargers	8-2
8.2.7	In-Line Filter	8-3
8.2.8	Injector	8-3
8.2.9	Column	8-3
8.2.10	Replacing the Check Valves	8-3
8.2.11	Piston Seal Replacement	8-9
8.2.12	Cam Wiper	8-22
8.2.13	Cutting Stainless Steel Tubing	8-23
8.2.14	Cutting Teflon Tubing	8-23
8.2.15	Stainless Steel Fittings	8-24

8. SERVICE AND MAINTENANCE

8.1. SERVICE

Service procedures other than those described below under Maintenance should be performed only by a Perkin-Elmer Service Engineer. Should service be required, please contact your local Perkin-Elmer representative.

8.2. MAINTENANCE

Routine maintenance is required to maintain the system in proper working condition. A list of recommended spare parts is included in the Installation Section of this manual.

8.2.1. Electrical Hazards

> **Warning:** High voltage is present at the power supply PC board in the Series 4 Solvent Delivery System. Before removing any panels, the Series 4 must be turned OFF and disconnected from the AC power source.

8.2.2. Mechanical Hazards

> **Warning:** The Series 4 Pump Module contains moving parts that could cause operator injury. Before attempting to perform routine maintenance or service, make sure that all motion is stopped and that the Series 4 is turned OFF and disconnected from the AC power source.

8.2.3. Routine Checks

(1) Check to see that the helium tank has sufficient pressure and that a spare tank is available if necessary.

(2) Check to see that the waste disposal bottle is not full and that the end of the drain tubing is inserted sufficiently.

(3) Check to see that there is sufficient solvent in the reservoirs and that the inlet filter is completely immersed in the solvent.

(4) Avoid spills and clean them up immediately should they occur.

8.2.4. Periodic Checks

The frequency of periodic maintenance required will be determined by the amount of use the system receives, the cleanliness of the environment and the number and nature of the samples being analyzed.

8.2.5. Solvent Input Filters

After a period of use, the 10 micron input filters may become partially clogged. If the solvent is water, the growth of micro-organisms may complicate the blockage. Plugged input filters can cause pump flow fluctuation.

To test the solvent input filters, in the Purge Mode, open the drain valve while the pump is not pumping (flow rate of 0). The solvent should flow freely out of the drain port. If it does not, the filters should be cleaned or replaced.

Air bubbles in the PTFE inlet tubing are a possible indication of a restriction which could cause pump flow fluctuation. Clean or replace the filter assembly (P-E Part No. 0993-7129). A cartridge kit containing two gaskets and two 10 µm filters (P-E Part No. 0254-0312) is available.

Clean the filter in an ultrasonic bath, isopropanol, or in mild nitric acid monthly. If mild nitric acid is used, make sure that all the acid is removed prior to reinstallation.

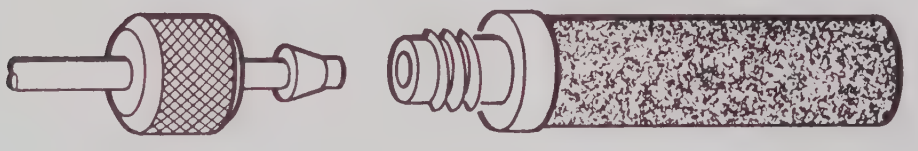


Figure 8-1 - Solvent Input Filter

8.2.6. Solvent Spargers

The solvent spargers should also be cleaned monthly to remove any surface accumulation. Clean the spargers by soaking in isopropanol alcohol.

Sparging is most effective when all reservoirs are filled to approximately the same level.

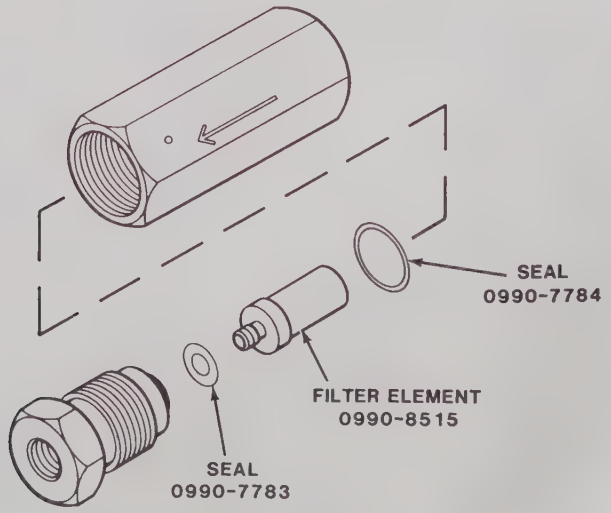


Figure 8-2 - In-Line Filter 0990-3606 (Detail)

8.2.7. In-Line Filter

The in-line 0.5 µm filter (P/N 0990-3606) located just after the drain valve and before the backpressure regulator should be cleaned periodically in an ultrasonic bath, isopropanol, or in mild nitric acid. If mild nitric acid is used, make sure that all the acid is removed prior to reinstallation. Replace the seals if necessary (see Figure 8-2). A cartridge kit for this filter is available as Part No. 0254-0311. It contains two filter element seals, two large filter element seals and two 0.5 µm filter cartridges.

8.2.8. Injector

Excessive solvent pressures at the pump outlet (pressures more than 3.45 MPa (500 psi) above the normal operating pressures for a given procedure) may indicate a partially clogged injector.

Remove the injector and clean it, following the instructions given in the Injector Operating Manual.

8.2.9. Column

Excessive pressures may also result from a clogged column inlet end fitting. Replace the column inlet end fitting, following the instructions packed with the column, or, if necessary, replace the column.

8.2.10. Replacing the Check Valves

Unusual variations in the pressure readout on the VDU display or a change or inconsistency in peak retention times may be an indication of check valve failure.

> **Important:** Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

Defective check valves should be replaced. There are two input check valves (P/N 0254-0177) and one output check valve (P/N 0254-0197) on the Series 4 Pump Head Assembly.

Rebuilt check valves are available on an exchange basis only from Perkin-Elmer. To obtain rebuilt check valves, send the defective check valve(s), accompanied by your purchase order showing the Perkin-Elmer part number for check valve(s) to

Perkin-Elmer Corporation
Supervisor, In-Plant Repair Facility,
Mail station 63
Main Avenue
Norwalk, CT 06856

If the defective valve does not accompany the purchase order, full price for a new check valve will be charged.

8.2.10.1. SLIDING THE PUMP MODULE ASSEMBLY FORWARD

(1) Open the door to the solvent pressurization chamber, turn the pressure regulator to zero and allow the solvent to depressurize.

(2) Turn off all power to the Series 4 and remove the power cord from the AC outlet.

(3) At the front of the Series 4, disconnect the outlet tubing at the bulkhead connector, using a 1/4" wrench (see Figure 8-3).

(4) To prevent siphoning, remove the caps and lines from the solvent reservoirs. Hold the caps while turning the bottles to avoid twisting the lines.

(5) Place the lines from the solvent reservoirs in a plastic bag and locate them out of the way.

(6) Remove the reservoirs from the solvent pressurization chamber.

(7) Remove the tray on the bottom of the solvent pressurization chamber.

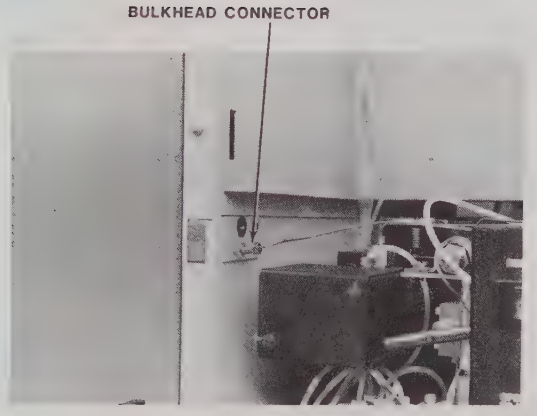


Figure 8-3a - Bulkhead Connector

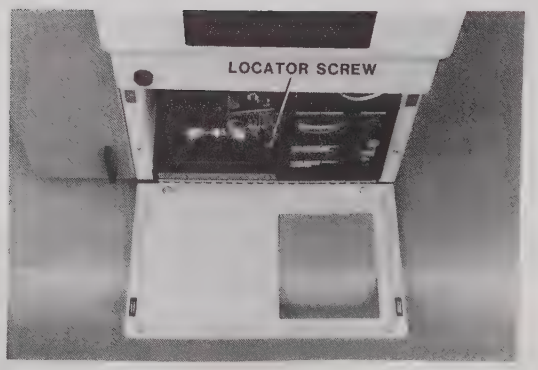


Figure 8-3b - Locator Screw

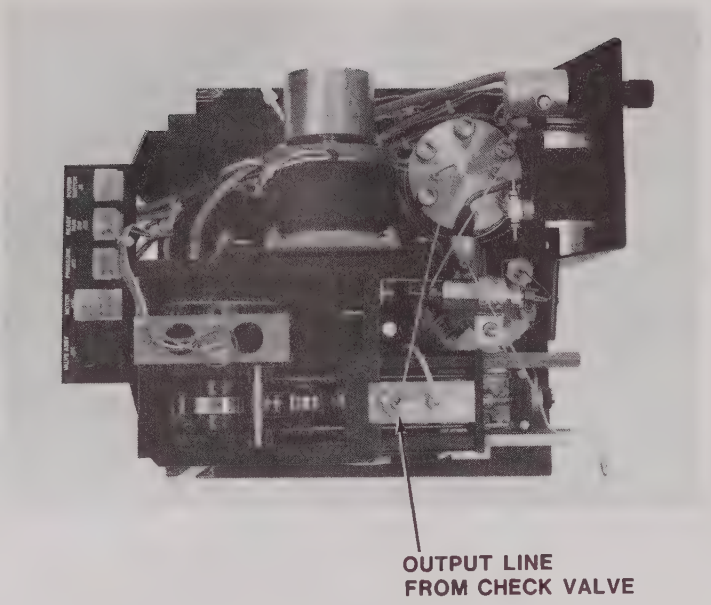


Figure 8-4 - Output Line From Check Valve (Pump Assembly) Removed from Instrument for Clarity)

(8) Disconnect the line from the output check valve (see Figure 8-4) to the pulse damper at the output check valve, using a 1/4" wrench.

(9) Unscrew the locator screw which is in the center front of the Pump Module Tray (see Figure 8-3).

The Pump Module will now slide forward out of the instrument on drawer-type tracks about 10 cm 4 inches, allowing access to the check valves.

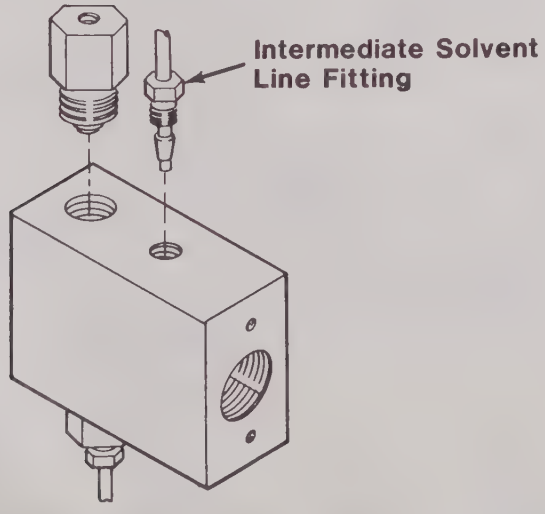


Figure 8-5 - Intermediate Solvent Line Fitting

8.2.10.2. REMOVING THE OLD CHECK VALVES:

(1) Use a 3/8" wrench to disconnect the intermediate solvent line.

(2) Use a 3/8" wrench to remove the input check valve line fittings.

> **Note:** Should a check valve loosen before the fitting, hold the check valve body with a 5/8" wrench while disconnecting the fitting from the check valve.

(3) Remove the line from the output valve to the pressure transducer, using a 1/4" wrench.

(4) Both the input and the output check valves are removed from the Pump Head assembly with a 5/8" wrench (see Figure 8-6).

The intermediate check valve is identical to the input check valve. Both the intermediate and the input check valves are located on the underside of the Pump Head. The input check valve is the one to the front of the instrument (see Figure 8-6).

> **Note:** We recommend using an inspection mirror to inspect the check valve port after removing the old check valve. Washers may split and all pieces should be removed.

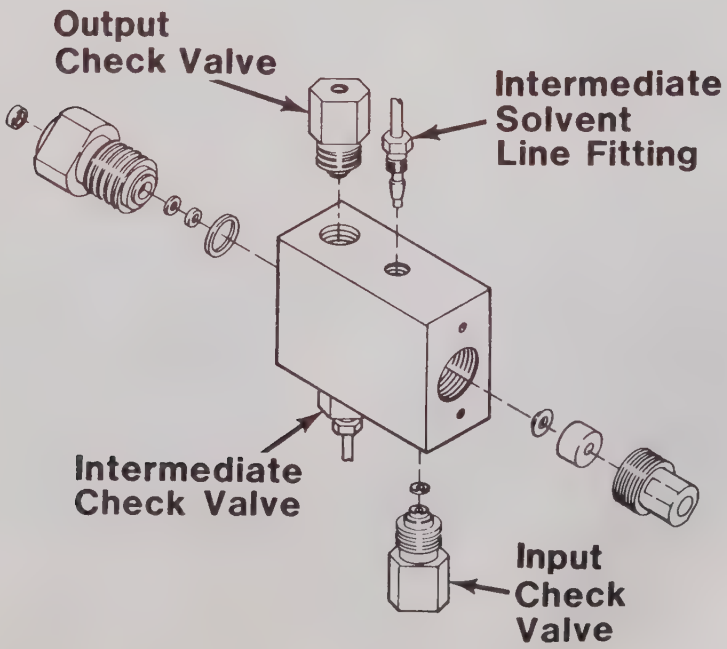


Figure 8-6 - Input and Output Check Valves

8.2.10.3. REPLACING WITH NEW CHECK VALVES:

Important: Do not use teflon pipe dope on check valve threads.

8.2.10.3.1. Input Check Valves (P/N 0254-0177)

The input check valves have a washer which should be sitting in the relief in the outlet end of the check valve (see Figure 8-7). Check to make sure that it is in place. If the washer is missing or damaged, it should be replaced. The washer is included with a new input check valve. It may also be ordered separately, part number 0254-2150.

(1) Replace the old check valve with the new check valve and tighten finger tight.

(2) Using a torque wrench with a 5/8" socket or crow's foot wrench, torque the check valve to 75 in./lbs. (85 kg/cm). Use Perkin-Elmer's Torque Wrench Kit, P/N 0254-0871, or the equivalent. When torquing, be sure to follow the instructions given with the torque wrench used (see Figure 8-8).

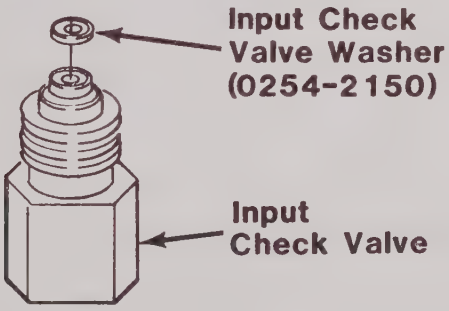


Figure 8-7 - Input Check Valve Washer

8.2.10.3.2. Output Check Valves (P/N 0254-0197)

The washer on the output check valve is an integral part of the valve.

(1) Replace the old check valve with the new check valve and tighten finger tight.

(2) Using a torque wrench with a 5/8" socket or crow's foot wrench, torque the check valve to 75 in./lbs. (85 kg/cm). Use Perkin-Elmer's Torque Wrench Kit, P/N 0254-0871, or the equivalent. When torquing, be sure to follow the instructions given with the torque wrench used. (See Figure 8-8.)

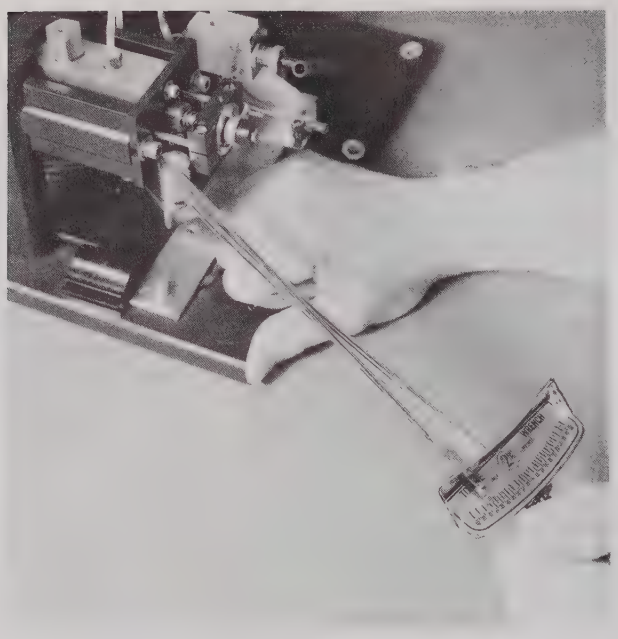


Figure 8-8 - Tightening Check Valves with a Torque Wrench

8.2.10.4. REINSERTING THE PUMP MODULE ASSEMBLY

(1) Reconnect the intermediate solvent line fittings using a 3/8" wrench.

(2) Reconnect the input solvent line fitting, using a 3/8" wrench.

(3) Slide the Pump Module back into the instrument.

(4) Tighten the locator screw into the hole in the center front of the frame.

(5) Reconnect the line from the output check valve to the pulse damper, using a 1/4" wrench.

(6) Reconnect the outlet tubing at the bulkhead connector, using a 1/4" wrench.

(7) Replace the tray on the bottom of the solvent pressurization chamber, making sure that the drain hole is in the rear right-hand corner of the pressure cabinet over the tygon tubing.

(8) Replace the solvent lines and fill and cap the reservoirs. Hold the caps in place and turn the reservoirs to avoid twisting the solvent lines.

Note: Do not fill the solvent reservoirs beyond the 1000 ml mark.

8.2.11. Piston Seal Replacement

Large system pressure fluctuations, low system hydraulic pressure or leaks in the system may indicate piston seal failure.

8.2.11.1. REPLACEMENT PARTS

Metering (low pressure) Piston Seal: 0990-7330

Metering (low pressure) Piston Guide: 0254-2313*

High Pressure Piston Seal: 0990-7324

High Pressure Piston Seal Back-up Ring: 0254-2076*

*Note: We strongly recomend that these be replaced when the associated Piston Seal is replaced.

Alternative seals for the high pressure piston are available for working with high pH:

Hi pH Seal: P/N 0990-7327
Heavy Duty Seal (usable at all pH): P/N 0990-7328

Important: Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

8.2.11.2 SLIDING THE PUMP MODULE ASSEMBLY FORWARD

(1) Open the door to the solvent pressurization chamber, turn the pressure regulator to zero and allow the solvent to depressurize.

(2) Turn off all power to the Series 4 and remove the power cord from the AC outlet.

(3) At the front of the Series 4, disconnect the outlet tubing at the bulkhead connector, using a 1/4" wrench (see Figure 8-3).

(4) Remove the caps and lines from the solvent reservoirs to prevent siphoning. Hold the caps while turning the bottles to avoid twisting the lines.

(5) Place the lines from the solvent reservoirs in a plastic bag or beaker and locate them out of the way.

(6) Remove the reservoirs from the solvent pressurization chamber.

(7) Remove the tray on the bottom of the sovlent pressurization chamber.

(8) Unscrew the locator screw which is in the center front of the Pump Module Tray (see Figure 8-3).

The Pump Module will now slide forward on drawer-type tracks about 4 inches, allowing access to the piston assemblies.

8.2.11.3. REMOVING THE PISTON ASSEMBLIES

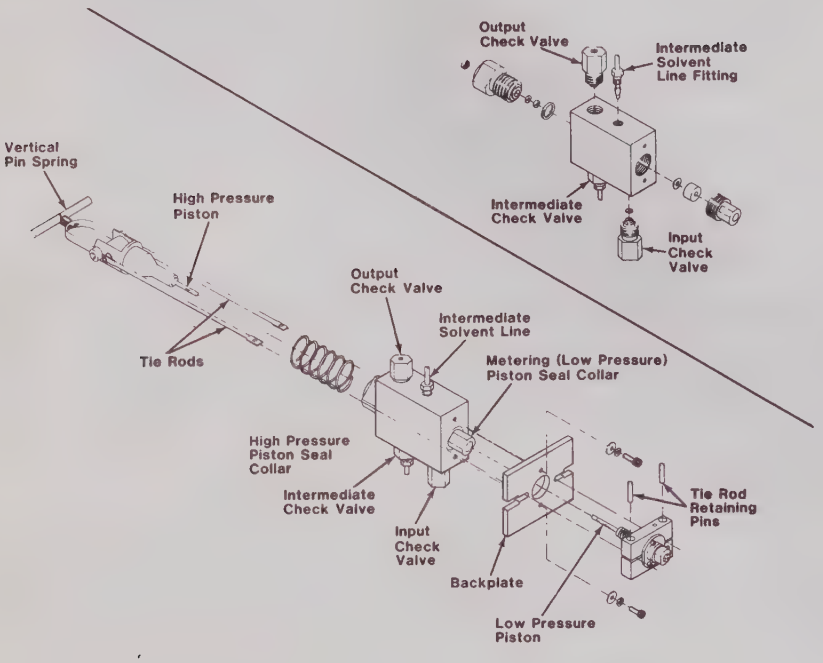


Figure 8-9 - Exploded View of Piston Assembly

8.2.11.3.1. Removing the Metering (Low Pressure) Piston

(1) Turn the drive belt by hand so that the cam rotates to its lowest point (see Figure 8-10). This will relieve as much tension as possible on the high pressure piston spring. The rear piston will jump backwards about 1/2" when it reaches the lowest point.

(2) Remove the 2 screws which hold the cover on the metering piston assembly. Slide the cover straight forward to remove it.

(3) Remove the 2 hexagonal standoffs on the black faceplate.

(4) Using a 1/4" wrench, remove the line from the output check valve to the pulse damper.

(5) Using a 3/8" wrench, remove the input check valve line.

(6) Use a 3/8" wrench to disconnect the intermediate solvent line at the upper fitting.

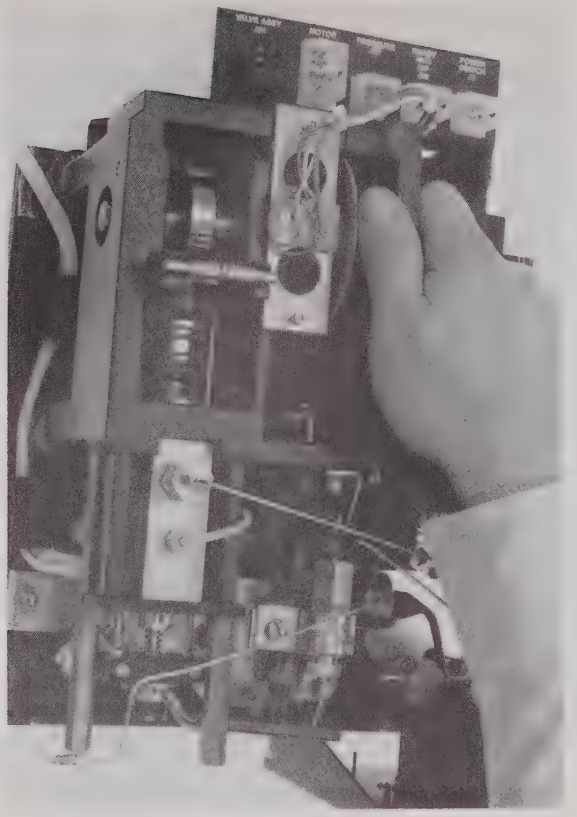


Figure 8-10 - Positioning The Cam (Pump Assembly removed from Instrument for Clarity)

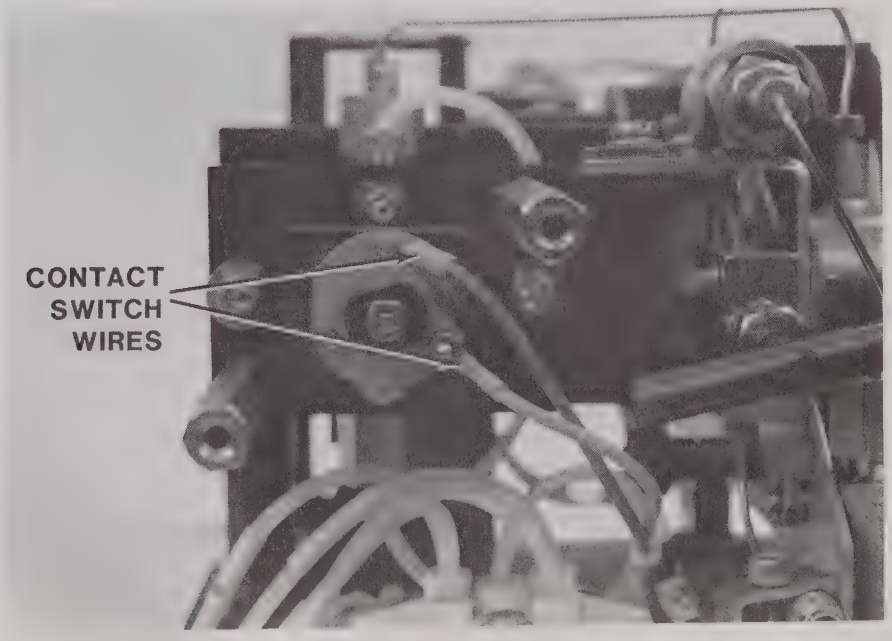


Figure 8-11 - Contact Switch Wires

(7) Disconnect the 2 wires from the contact switch on the front of the metering (lower pressure) piston block. (This step is not necessary but may make the task easier and will allow the piston to be placed out of the way.) (See Figure 8-11.)

(8) If the 2 wires have been removed from the contact switch, place the metering (low pressure) piston on a smooth surface away from the immediate work area where it will not be accidentally damaged.

(9) Two retaining pins hold the tie rods in place in the metering (low pressure) piston block (see Figure 8-12). Use an Allen wrench or short length of 1/16" stainless tubing or other appropriate tool to push the pins up from the bottom so that they may be lifted out.

> **Important:** The pistons are made of synthetic sapphire and are extremely fragile. Support the piston carefully and slide it straight out when removing it. Side-loading pressure may break the piston.

(10) Use a 5/32" Allen wrench to remove the left hand Allen screw (see Figure 8-13) which holds the Pump Head in place. Place the index finger of one hand behind the casting and hold the thumb in front of the black cover plate as shown in Figure 8-14.

Note: If left-handed, you may want to reverse the left-right sequence given.

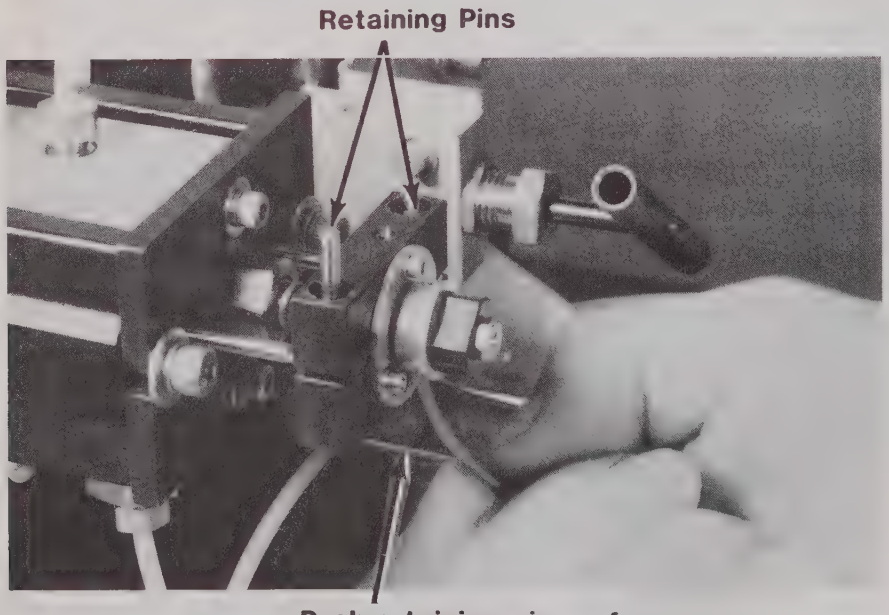


Figure 8-12 - Retaining Pins

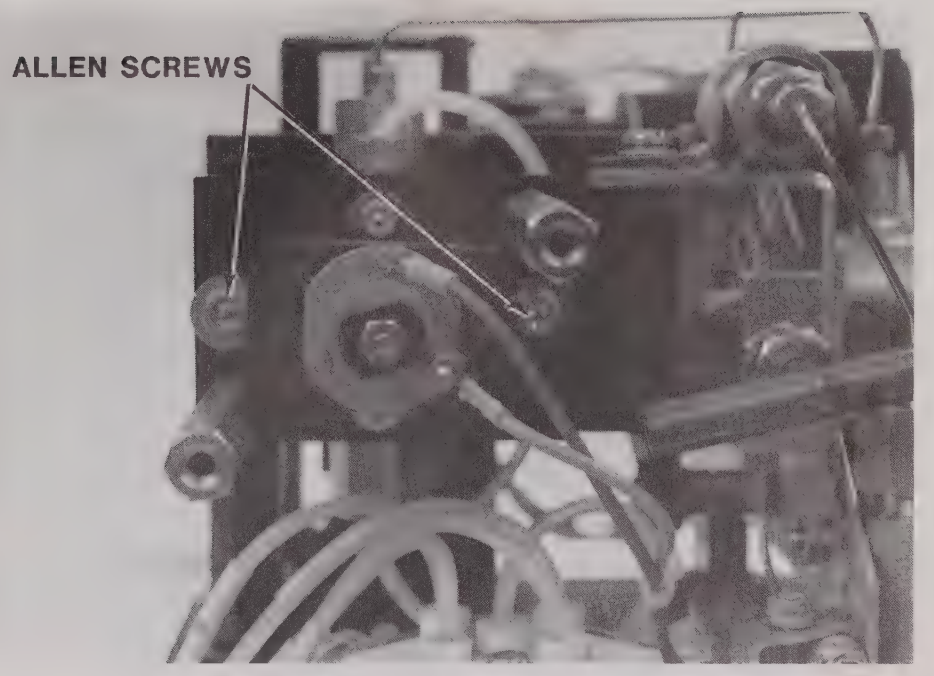


Figure 8-13 - Allen Screw Location

Figure 8-14 - Supporting the Pump Head

(11) When the second screw is removed, gradually release the thumb pressure and allow the Pump Head Assembly to jump out approximately 3/4".

(12) Hold the Pump Head Assembly firmly and pull it straight forward slowly to keep from breaking the High Pressure piston. Be careful not to snag the Pump Head Assembly on the solvent input lines.

If only the metering (low pressure) piston seal is to be changed, go directly to the steps listed below under Removing and Replacing the Seals.

8.2.11.3.2. Removing the High Pressure Piston

(1) Figure 8-15 shows the spring pin held across the top of the casting by a vertical spring which provides vertical tension on the piston.

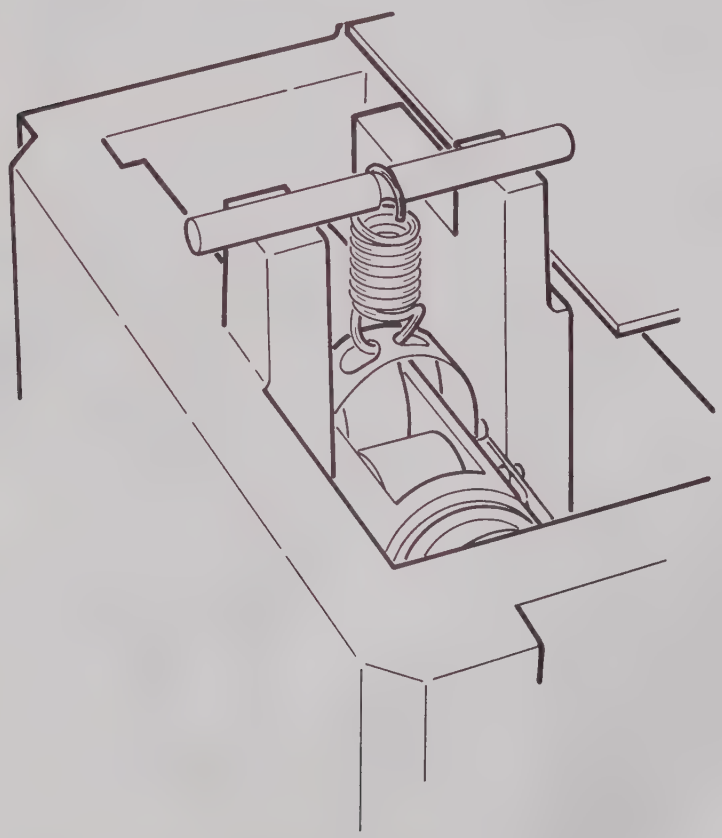


Figure 8-15 - Vertical Spring Pin

Figure 8-16 - Freeing The Vertical Spring Pin

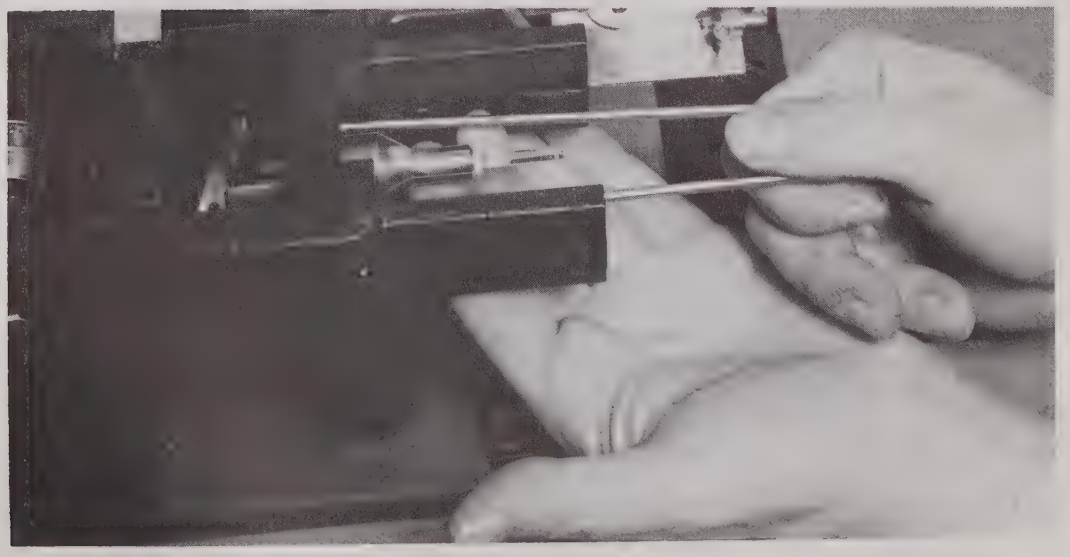


Figure 8-17 - Removing the High Pressure Piston

To release the pin, hold the thumb and forefinger of the right hand as shown in Figure 8-16. In this position, the thumb may be used to push, while the index finger is used to pull on the pin. A quick twisting, snapping motion will free the pin and relieve the upward pressure on the piston.

(2) Remove the pin.

(3) While gently pulling straight forward on the tie rods, (see Figure 8-17) apply a slight inward pressure on the rods while pulling forward will help to support the piston.

> **Important:** The pistons are made of synthetic sapphire and are extremely fragile. Support the piston carefully and slide it straight out when removing it. Side-loading pressure may break the piston.

(4) Place the piston assembly on a smooth surface away from the immediate work area where it will not be accidentally damaged.

8.2.11.4. INSPECTING AND CLEANING THE PISTONS

Visually inspect for scratches.

> **Note:** Subsurface imperfections can appear to be scratches.

If scratches cannot be removed by cleaning, the piston should be replaced.

Clean the piston with toothpaste (Pepsodent or equivalent; a toothpaste with high diatomateous earth content). Thoroughly flush the piston with distilled water or wipe it with a tissue which has been saturated with water to rinse off the toothpaste.

8.2.11.5 REMOVING AND REPLACING THE PISTON SEALS

8.2.11.5.1. Metering (low pressure) Piston Seal

(1) Remove the black face plate from the Pump Head Assembly.

(2) Place the Pump Head Assembly in a bench vise, if available. Otherwise, use a 1" wrench to hold the Pump Head Assembly.

(3) Use a 1/2" wrench to remove the Metering (low pressure) piston seal collar. If a 1" wrench is used to hold the Pump Head Assembly, orient the two wrenches with the narrowest angle possible between them and squeeze the two wrenches together to break the torque as shown in Figure 8-18.

(4) Remove the seal from the Pump Head with a small clean tool.

(5) Place the new seal in the pump head.

(6) Seat the new Metering (low pressure) piston seal by pressing in with the thumb and replacing the Piston Seal Collar (Figure 8-19).

(7) Tighten the Piston Seal Collar using the 1/2" wrench.

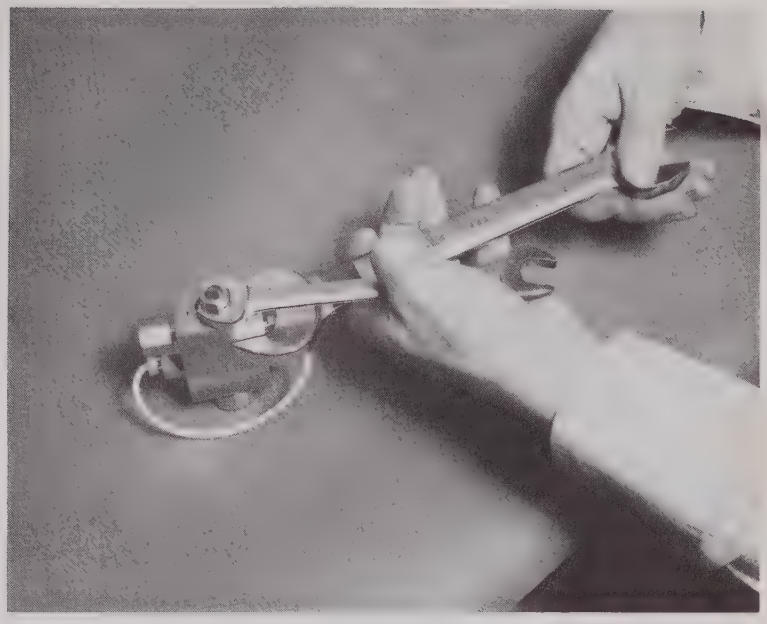


Figure 8-18 - Removing Piston Seal Collar

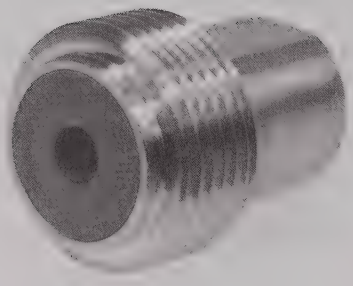


Figure 8-19 - Metering (low pressure) Piston Guide Bushing Inside Piston Seal Collar

8.2.11.5.2. High Pressure Piston Seal

(1) Place the Pump Head Assembly in a bench vise, if available. Otherwise, use a 1" wrench to hold the Pump Head Assembly.

(2) Use a 5/8" wrench to remove the high pressure piston seal collar. If a 1" wrench is used to hold the Pump Head Assembly, orient the two wrenches with the narrowest angle possible between them and squeeze the two wrenches together to break the torque as shown in Figure 8-18.

(3) Use the end of the seal remover (0254-2077) or other small clean tool to carefully push out the old seal and back-up ring (see Figure 8-20).

Note: We strongly recommend that these be replaced as a pair.

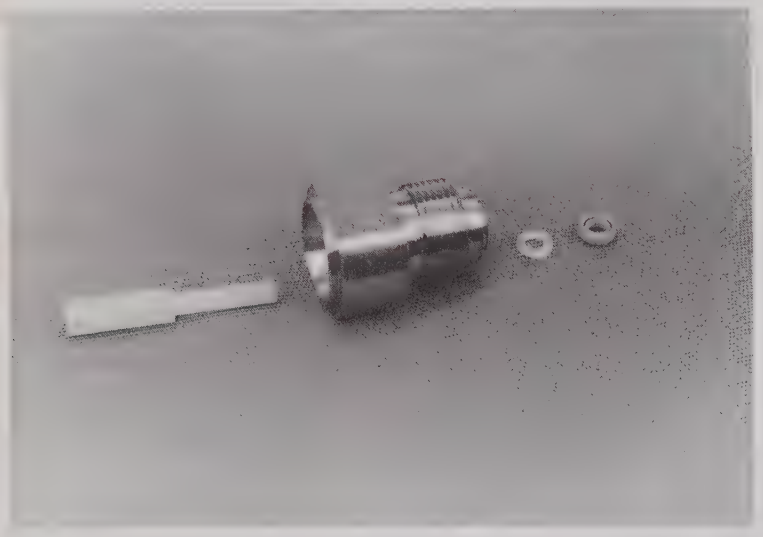


Figure 8-20 - High Pressure Piston Seal and Back-Up Ring

(4) Place the new seal and the new back-up ring in the Pump Head.

(5) Press the new seal firmly into place with your thumb.

(6) Replace the High Pressure Piston Seal Collar.

(7) Tighten the Piston Seal Collar using the 5/8" wrench.

8.2.11.6. REPLACING THE PISTON ASSEMBLIES

8.2.11.6.1. Replacing the High Pressure Piston and Pump Head

(1) Reinstall the black face plate on the Pump Head.

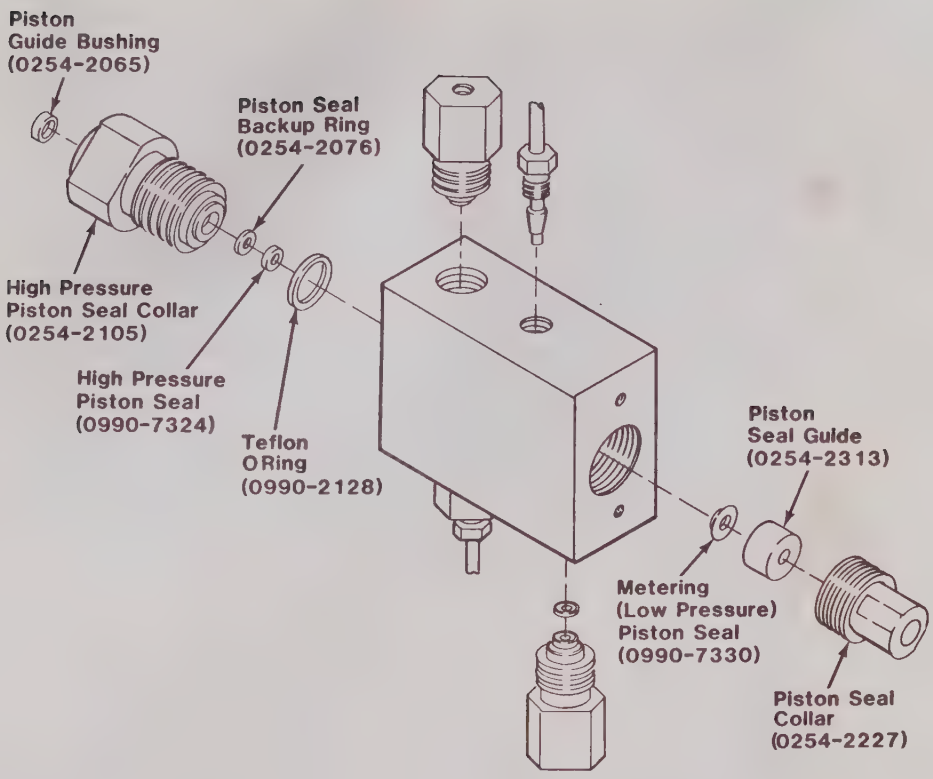


Figure 8-21 - Piston Seals - Exploded View

(2) With one hand, hold the High Pressure Piston Assembly by applying a slight inward pressure to the tie rods while guiding the piston assembly into place in the casting with the other hand.

> **Important:** Be careful not to get any grease on the surface of the piston. Make sure that the roller bearings on the sides of the tie rods fit properly into the tracks. A slight upward pressure on the vertical spring will help to position the piston. Gently guide the piston into place with the tie rods.

Note: The High Pressure Piston Assembly may go in either way. There is no "right side up."

(3) Place the large compression spring around the piston body.

(4) Make sure that the lower hook of the vertical spring is passed from one hole in the spring holder to the other and that it does not pass through one hole and around the outside of the holder. When properly assembled, the hooks on the vertical spring will be at right angles to each other (see Figure 8-22).

(5) Place the pin through the upper hook of the vertical spring and postion the right hand as shown in Figure 8-23. While the left hand continues to support the tie rods, the thumb and forefinger of the right hand as used to twist the pin and snap it into place on its supports.

(6) Carefully guide the reassembled Pump Head Assembly over the piston, making sure that the tie rods are in the slots on the black cover plate.

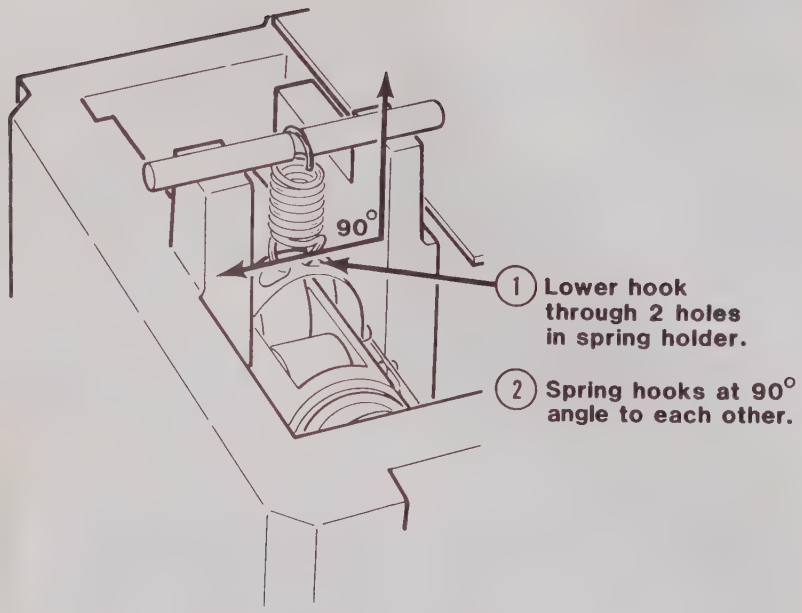


Figure 8-22 - Correct Positioning of Hooks on Vertical Spring

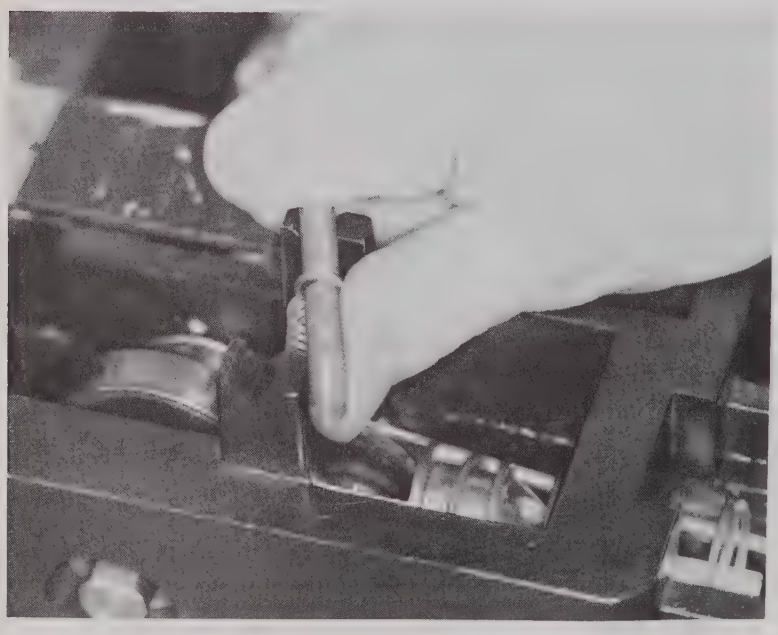


Figure 8-23 - Replacing the Vertical Pin Spring

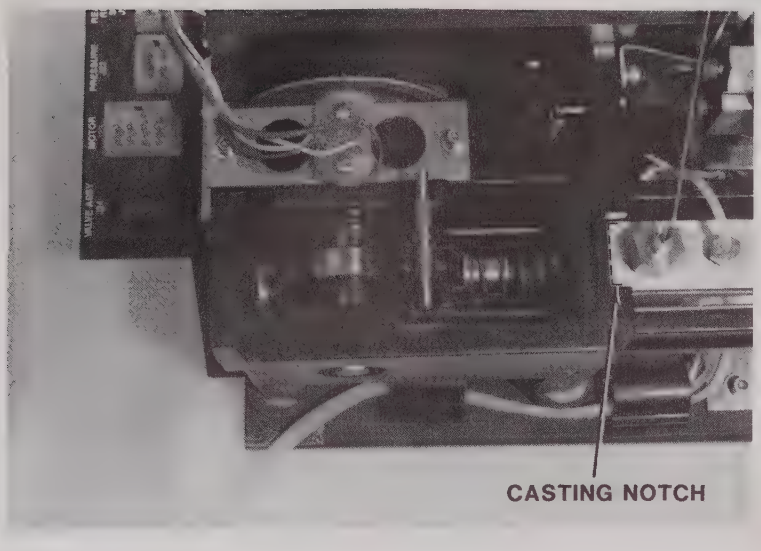


Figure 8-24 - Proper Positioning of Pump Head In Casting Notch (Assembly removed from Instrument for Clarity)

(7) Check the alignment carefully. When the Pump Head Assembly is tightened in place, the relief on the piston seal collar must go inside the coil of the spring. the pump head must fit snugly and squarely in the notch in the casting (see Figure 8-24).

(8) Holding the Pump Head Assembly firmly in place with the thumb and forefinger of one hand, as shown in Figure 8-24, replace the right-hand Allen screw and tighten finger-tight. Then replace the left-hand Allen screw and tighten finger-tight. Release the Pump Head Assembly. Then tighten each Allen screw in an alternating fashion, making sure that even torque is maintained. Continue until the Pump Head fits snugly and squarely in the notch in the casting.

8.2.11.6.2. Replacing the Metering (low pressure) Piston

(1) Carefully insert the Metering (low pressure) Piston in the piston seal collar, lining up the holes in the tie rods with the holes in the Metering Piston Assembly.

(2) Replace the tie rod pins.

(3) Reconnect the line from the output check valve to the pulse damper, using a 1/4" wrench.

(4) Reconnect the input check valve line and intermediate solvent line, using a 3/8" wrench.

(5) If disconnected, reconnect the two wires to the contact switch on the front of the Metering Piston Assembly.

(6) Replace the standoffs which hold the Metering Piston Assembly cover.

(7) Replace the Metering Piston Assembly cover, making sure that the slot for the wires is on the lower right-hand side.

(8) Slide the Pump Module back into the instrument.

(9) Tighten the locator screw into the hole in the center front of the frame.

(10) Reconnect the outlet tubing at the bulkhead connector, using a 1/4" wrench.

(11) Replace the tray on the bottom of the sovlent pressurization chamber, making sure that the drain hole is in the rear right-hand corner of the pressure cabinet over the tygon tubing..

(12) Replace the solvent lines and fill and cap the reservoirs. Hold the caps in place and turn the reservoirs to avoid twisting the solvent lines.

Note: Do not fill the sovlent reservoirs beyond the 1000 ml mark.

8.2.12. Cam Wiper

Once a year, or more frequently, depending upon use, apply approximately 1/4 oz. (6 or 7 cc) of Molykote grease (P/N 0990-8511) to the white felt cam wiper located on the underside of the cam.

The wiper is accessible through a hole in the rear of the Pump casting (see Figure 8-25).

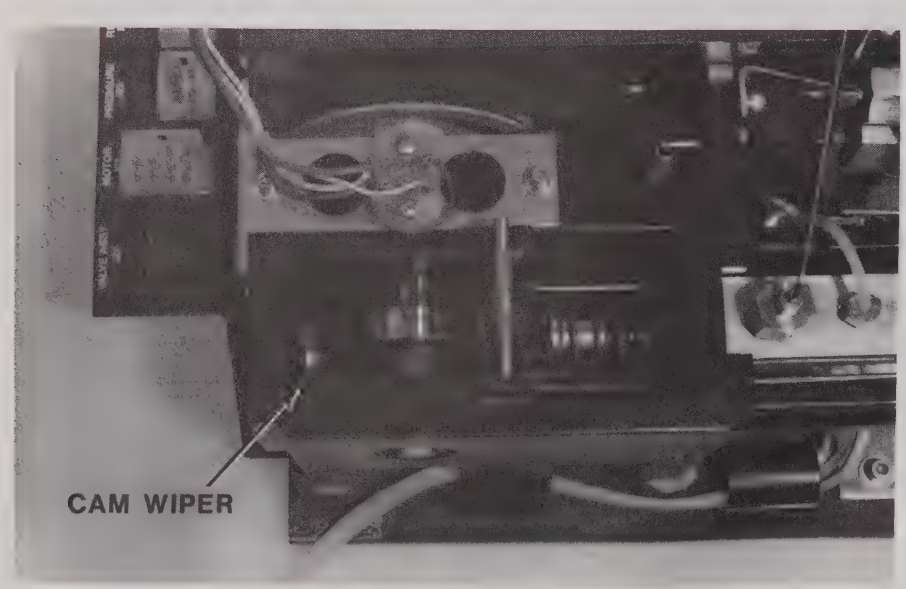


Figure 8-25 - Cam Wiper Location

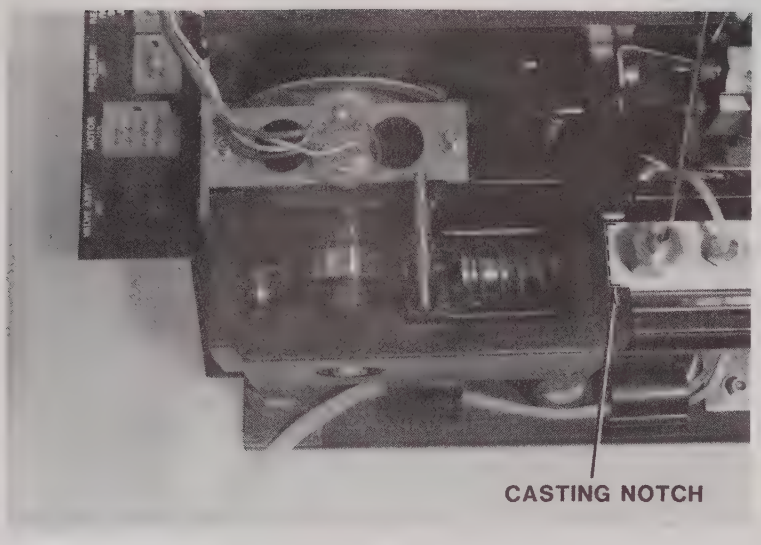


Figure 8-24 - Proper Positioning of Pump Head In Casting Notch (Assembly removed from Instrument for Clarity)

(7) Check the alignment carefully. When the Pump Head Assembly is tightened in place, the relief on the piston seal collar must go inside the coil of the spring. the pump head must fit snugly and squarely in the notch in the casting (see Figure 8-24).

(8) Holding the Pump Head Assembly firmly in place with the thumb and forefinger of one hand, as shown in Figure 8-24, replace the right-hand Allen screw and tighten finger-tight. Then replace the left-hand Allen screw and tighten finger-tight. Release the Pump Head Assembly. Then tighten each Allen screw in an alternating fashion, making sure that even torque is maintained. Continue until the Pump Head fits snugly and squarely in the notch in the casting.

8.2.11.6.2. Replacing the Metering (low pressure) Piston

(1) Carefully insert the Metering (low pressure) Piston in the piston seal collar, lining up the holes in the tie rods with the holes in the Metering Piston Assembly.

(2) Replace the tie rod pins.

(3) Reconnect the line from the output check valve to the pulse damper, using a 1/4" wrench.

(4) Reconnect the input check valve line and intermediate solvent line, using a 3/8" wrench.

(5) If disconnected, reconnect the two wires to the contact switch on the front of the Metering Piston Assembly.

(6) Replace the standoffs which hold the Metering Piston Assembly cover.

(7) Replace the Metering Piston Assembly cover, making sure that the slot for the wires is on the lower right-hand side.

(8) Slide the Pump Module back into the instrument.

(9) Tighten the locator screw into the hole in the center front of the frame.

(10) Reconnect the outlet tubing at the bulkhead connector, using a 1/4" wrench.

(11) Replace the tray on the bottom of the sovlent pressurization chamber, making sure that the drain hole is in the rear right-hand corner of the pressure cabinet over the tygon tubing..

(12) Replace the solvent lines and fill and cap the reservoirs. Hold the caps in place and turn the reservoirs to avoid twisting the solvent lines.

Note: Do not fill the sovlent reservoirs beyond the 1000 ml mark.

8.2.12. Cam Wiper

Once a year, or more frequently, depending upon use, apply approximately 1/4 oz. (6 or 7 cc) of Molykote grease (P/N 0990-8511) to the white felt cam wiper located on the underside of the cam.

The wiper is accessible through a hole in the rear of the Pump casting (see Figure 8-25).

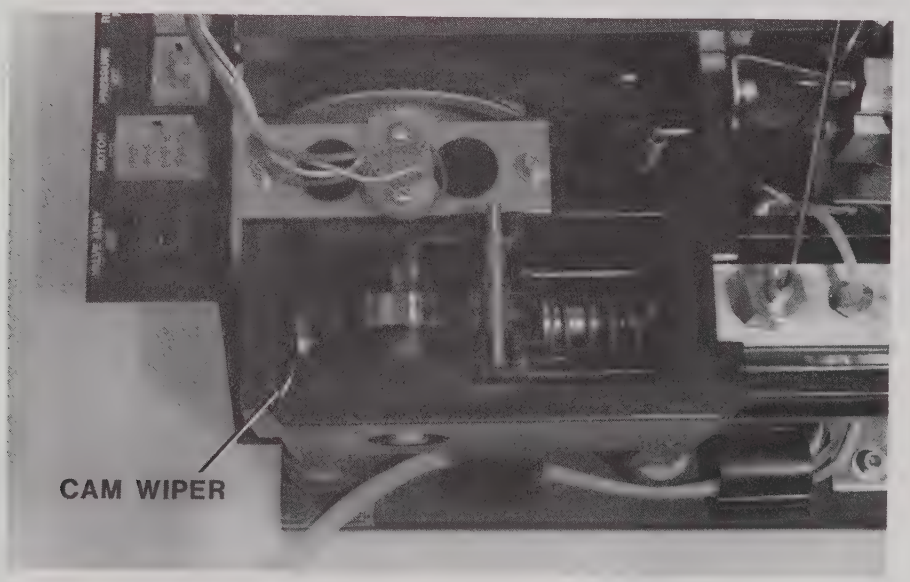


Figure 8-25 - Cam Wiper Location

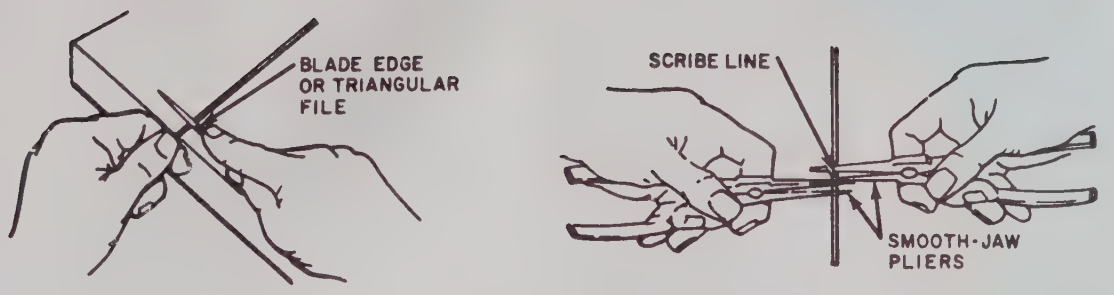


Figure 8-26 - Cutting Stainless Steel Tubing

8.2.13. Cutting Stainless Steel Tubing

Tubing should be scribed with a triangular or knife-edge file and then held firmly with two pairs of smooth-jaw pliers and bent back and forth to break.

> **Note:** These instructions for cutting stainless steel tubing are for the large I.D. tubing only. Special care is needed when cutting the 0.007 tubing. See the Installation Section of this manual for lengths of percut 0.007 tubing available.

8.2.14. Cutting Teflon Tubing

The Teflon tubing should be cut to the proper length with a razor blade, making sure that the cut is at a 90° angle. To grip the tubing firmly, use a piece of very fine sand paper (#400-#600 grit) to hold the tubing about 1/2 inch (2 cm) from the end.

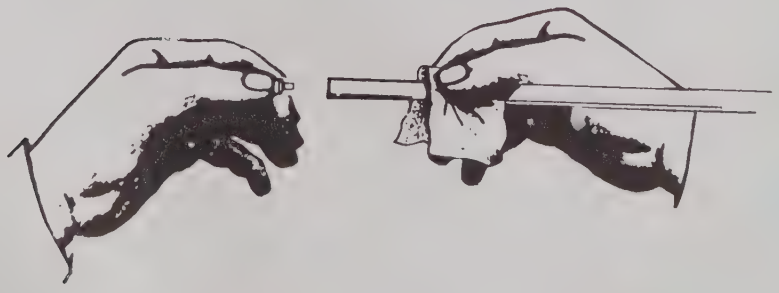


Figure 8-27 - Gripping Teflon Tubing and Inserting the Ferrule

(1) Flare the end of the tubing slightly with a hot soldering iron (25 watt) with a conical tip so that the ferrule may be inserted.

(2) Press in the ferrule.

(3) Bring up the fitting, but do not push the fitting all the way to the ferrule. Leave it about 1/8" from the end. when the fitting is tightened, it will snug up. If you attempt to push the fitting to the bottom, the tubing may kink.

8.2.15. Stainless Steel Fittings

The stainless steel fittings in the Series 4 are Parker Hannifin fittings. These fittings are shown in Figure 8-28.

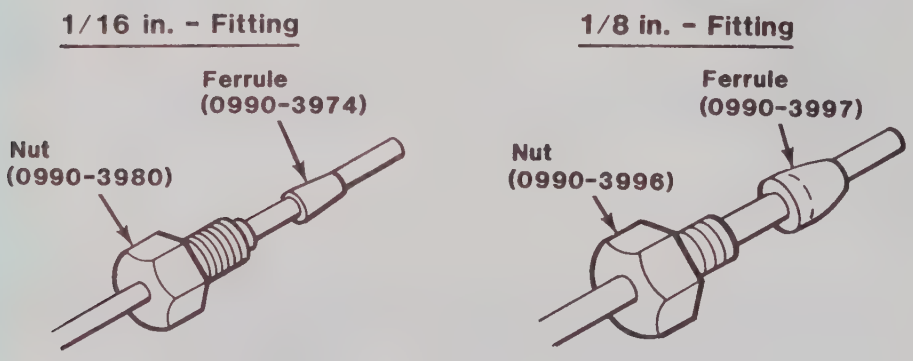


Figure 8-28 - Parker-Hannifin Fittings

9 INSTALLATION

Section		Page
9.1	Unpacking	9-1
9.2	Parts Supplied	9-1
9.3	Series 4 Start-Up Kit	9-2
9.4	Series 4 Accessories Available	9-2
9.5	Series 4 Optional Maintenance Kit	9-3
9.6	Series 4 Spare Parts Available	9-3
9.7	Laboratory Requirements	9-4
9.7.1	Dimensions	9-4
9.7.2	Environment	9-5
9.7.3	Configuration	9-8
9.7.4	Electrical Requirements	9-8
9.8	Connections	9-8
9.9	Power Connection	9-8
9.9.1	Voltage Selection & System Fuse	9-8
9.9.2	Power Cord	9-10
9.9.3	Power Supply Fuse	9-10
9.10	Helium Supply	9-10
9.11	Optional Vent Line	9-10
9.12	Connections to Injector	9-12
9.13	Connections to Column	9-12
9.14	Connections to Detector	9-12
9.15	Connections to the ISS-100 Autosampler	9-13
9.16	Connection to the 420B Autosampler	9-13
9.17	Adjusting the Pulse Damper Sealing Screw	9-20
9.17.1	Installation	9-20
9.17.2	Storage or Reshipment	9-20
9.18	Set-Up of Series 4 Pump Module	9-22
9.19	Set-Up of the Control Module	9-23
9.19.1	Data Line Switches	9-23
9.19.2	Setting the Parity and the Baud Rate on the Series 4 Pump Module	9-24
9.20	Degassing (Sparging) the Solvents	9-24
9.21	Pressurizing the Solvents	9-24
9.22	Priming the Pump and Purging the System	9-24

9. INSTALLATION

Installation of the Series 4 Solvent Delivery System is to be performed only by a Perkin-Elmer Service Engineer.

9.1. UNPACKING

The Series 4 Pump Module is shipped in one box, the Control Module is shipped in a second box, and, if ordered, the Series 4 Column Oven is shipped in a third box. The Series 4 Pump Module is shipped completely assembled. The 4 solvent reservoirs are contained in a separate box inside the Series 4 box. The Start-Up Kit is also in a separate box inside the Series 4 box. Any accessories ordered with the Series 4 Solvent Delivery System will be shipped in their own containers. When unpacking, check the items against the packing list. Remove all styrofoam and other packing material. Check for any signs of obvious shipping damage. Report any damage or missing items to the carrier and to the Perkin-Elmer Corporation immediately.

9.2. PARTS SUPPLIED

	117 VAC*	234 VAC
Series 4 Solvent Delivery System Series 4 Pump Module and Control Module Series 4 Start-Up Kit	0254-0164	0254-0165
Series 4 Solvent Delivery System with #7125-S Valve Series 4 Pump Module and Control Module #7125-S Valve Series 4 Start-Up Kit	0254-0295	0254-0296
Series 4 Solvent Delviery System with #7125-S Valve and Column Oven Series 4 Pump Module and Control Module #7125-S Valve Column Oven Series 4 Start-Up Kit	0254-0297	0254-0298
Series 4 Pump Module Only (without Control Module) Series 4 Pump Module Series 4 Start-Up Kit	0254-0283	0254-0284
Series 4 Pump Module Only (without Control Module) with #7125-S Valve Series 4 Pump Module #7125-S Valve Series 4 Start-up Kit	0254-0291	0254-0292
Series 4 Pump Module Only (without Control Module) with #7125-S Valve and Column Oven Series 4 Pump Module #7125-S Valve Column Oven Series 4 Start-Up Kit	0254-0285	0254-0286

*110 VAC is accommodated with this part number

9.3. SERIES 4 START-UP KIT, PART NUMBER 0254-0181

The Series 4 Start-Up Kit, which is shipped with every Series 4 Solvent Delivery System, contains the following items:

Part No.	Description	Quantity
0254-2267	Injector Mounting Bracket	1
0990-3974	1/16" CPI Ferrule	4
0990-3980	1/16" CPI Nut	1
0087-9078	30 ml Syringe	1
0254-2076	Seal Backup Ring (High Pressure)	2
0254-2077	Back Up Ring Insertion Tool	1
0254-2396	Tube (Pump to Injector Valve)	1
0254-2313	Seal Guide (Low Pressure)	1
0990-7324	Seal-High Pressure	2
0990-7330	Seal-Low Pressure	2
0990-7278	5/32 Allen Key	1
0090-7271	3/32 Allen Key	1
0254-2384	Grip, Teflon Tube	1
0993-9731	Series 4 Instruction Manual	1
59300-0043-00	LC Terminal User's Manual*	1
T55-130	RS-232 Cable *	1
0990-3129	Ferrule, Front	1
0990-3130	Ferrule, Back	1
0254-2188	Nut, Gland, 1/8 Tube	1
0990-3990	Ferrule, 1/8 Tube	1
0990-3128	Nut	1
-	Tubing, TFE, 0.060" ID x 0.125"OD	8 ft.
0254-2415	Vent Line Cap	3
0254-2416	Helium Input Line Cap	3

*These items are packed with the LC Terminal

9.4. SERIES 4 ACCESSORIES AVAILABLE

Part No.	Description	Quantity
0254-0196	Series 4 Column Oven	1
0254-2383	GPC Oven Inserts (each - 2 required for each 30 cm x 11 mm volume)	1
0254-2267	Injector Panel (blank)	1
0254-0140	7125-S Valve and Tubing	1
0254-0141	Standard 7125 Valve and Tubing	
0254-0142	Stop Flow Column Switching Valve with Tubing and Instructions	1
0990-3607	7125-S High Speed Valve (Valve only)	1
0990-3591	Standard 7125 Valve (Valve only)	1
0254-1647	Solvent Tray	1
0990-4937	10 µl Syringe	1
0254-0230	Prep Column Clamp Kit (Not for Use with Oven)	1
0089-0839	Solvent Filtration Kit - Aqueous	1

Part No.	Description	Quantity
0990-4986	Solvent Filter Replacement Kit - Aqueous	1
0089-0841	Solvent Filtration Kit - Organic	1
0990-4987	Solvent Filter Replacement Kit - Organic	1
0089-0835	Sample Filtration Kit - Aqueous	1
0089-0836	Sample Filter Replacement Kit - Aqueous	1
0089-0837	Sample Filtration Kit - Organic	1
0089-0838	Sample Filter Replacement Kit - Organic	1
0990-4992	Replacement Filter Holder Parts	1
0023-0090	Helium Regulator	1
0090-3401	Barbed Fitting for Vent Line	1

9.5. SERIES 4 OPTIONAL MAINTENANCE KIT, PART NUMBER 0254-0290

The Series 4 Optional Maintenance Kit contains supplies, tools and cables needed for an estimated period of 1 year of operation.

Part No.	Description	Quantity
0254-0943	Marker Interconnection Cable	1
0254-2076	Piston Seal Back-up Ring (High Pressure)	2
0254-2150	Check Valve Sealing Washer	2
0254-2313	Seal Guide (Low Pressure)	2
0254-2384	Grip, Teflon Tube	2
0271-0337	Recorder Cable (Pressure Monitoring)	1
0990-2128	O-Ring (large)	2
0990-3996	Inverted (1/8") Tube Nut	2
0990-3997	(1/8") Tube Ferrule	2
0990-7239	Wrench-Open End (1/2" - 9/16")	1
0990-7237	Wrench (1/4" - 5/16")	1
0990-7274	Wrench-Open End (5/8" - 3/4")	1
0990-7324	Seal-High Pressure	2
0990-7330	Seal-Low Pressure	2
0990-8511	Grease-Molykote (15 oz. can)	1
0990-3980	1/16" CPI Nut	4
0990-3974	1/16" CPI Ferrule	4
0990-2034	Reservoir O-Ring	4
0998-1616	4.0A 250V Slo-blo Fuse (234 VAC)	2
0998-1626	3A 250V Slo-blo Fuse (117 VAC)	2
0998-1761	6.25A 250V Slo-blo Fuse (117 VAC)	2
0998-1776	1.5A 250 Slo-blo Fuse	2

9.6. SERIES 4 SPARE PARTS AVAILABLE

Part No.	Description	Quantity
0254-0838	0.007 I.D. x 1/16" O.D. Stainless Tubing	6 ft.
0087-3035	0.043 I.D. x 1/16" O.D. Stainless Tubing	6 ft.
0087-3036	0.015" I.D. x 1/6" O.D. Stainless Tubing	6 ft.
0089-1479	0.007 I.D. x 1/16" O.D. Stainless Tubing	7.5 cm

Part No.	Description	Quantity
0089-1441	0.007 I.D. x 1/16" O.D. Stainless Tubing	20 cm
0089-1480	0.007 I.D. x 1/16" O.D. Stainless Tubing	50 cm
	1/16" I.D.x 1/8" O.D.Teflon Tubing (Solvent Lines)	42 inch
0990-3990	Kel-F Ferrule	4
0254-2188	Polypropolene Nut	4
0990-4811	Solvent Reservoir	1
0254-0176	High Pressure Piston	1
0254-2149	Low Pressure (Metering) Piston	1
0254-0177	Input Check Valve (2 per Series 4)	1
0254-0197	Output Check Valve (1 per Series 4)	1
0993-7129	Input Solvent Filter	1
0990-3606	In-Line Solvent Filter	1
0254-0871	Torque Wrench	1
0993-9824	Series 4 Service Manual	1
0993-9821	Series 4 Illustrated Parts List	1
0990-3590	Sparger	1
0254-0272	Backpressure Regulator	1
0254-2076	Back-Up Ring (High Pressure Seal)	1
0254-2150	Check Valve Sealing Washer	1
0990-7324	Seal, High Pressure	1
0990-7330	Seal, Low Pressure	1
0990-7327	Hi pH Seal	
0990-7328	Heavy Duty Seal	1

9.7. LABORATORY REQUIREMENTS

9.7.1. Dimensions

The dimensions of the Series 4 Pump Module are shown in Figure 9-1a. The weight of the Series 4 Pump Module is 28.4 kg. (62.5 pounds). The dimensions of the Control Module are shown in Figure 9-1b. The weight of the Contorl Module is 13.6 kg. (30 pounds). The Optional Column oven weighs 10.2 kg (22.5 lbs.). The bench must support this weight as well as the weight of the other items being used in the system.

Additionally, a mimimum of 5 cm (2 inches) should be provided at the rear of the instrument for air circulation and cable clearance.

9.7.2. Environment

The Series 4 should be placed in a clean environment with relatively stable temperature and humidity. The environment should be free from excessive vibration and strong electrical and magnetic fields.

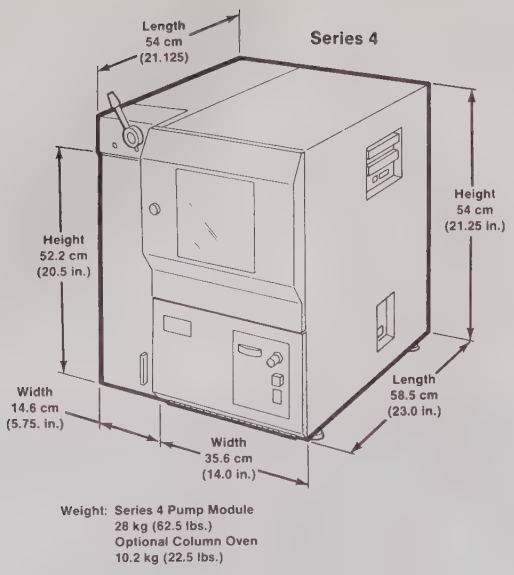


Figure 9-1a - Dimensions of Series 4 Pump Module with Optional Column Oven.

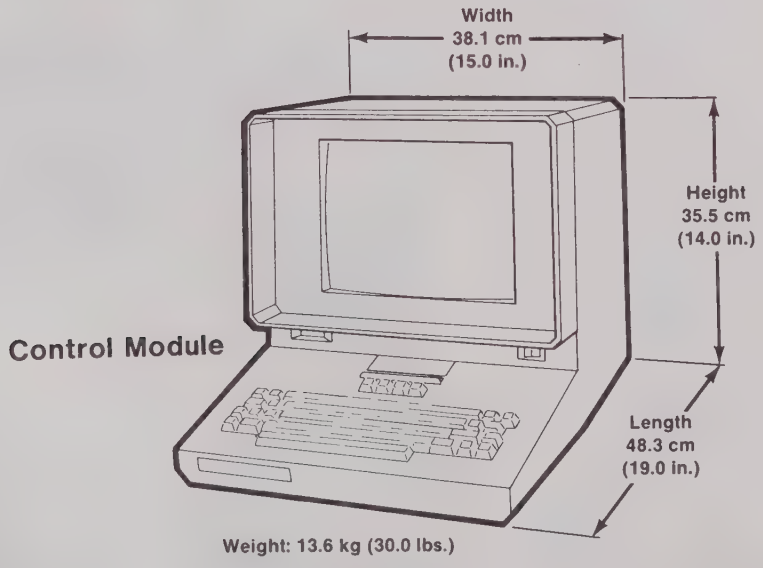


Figure 9-1b - Dimensions of Series 4 Control Module

Figure 9-2 - The Series 4 Solvent Delivery System with the ISS-100 Autosampler

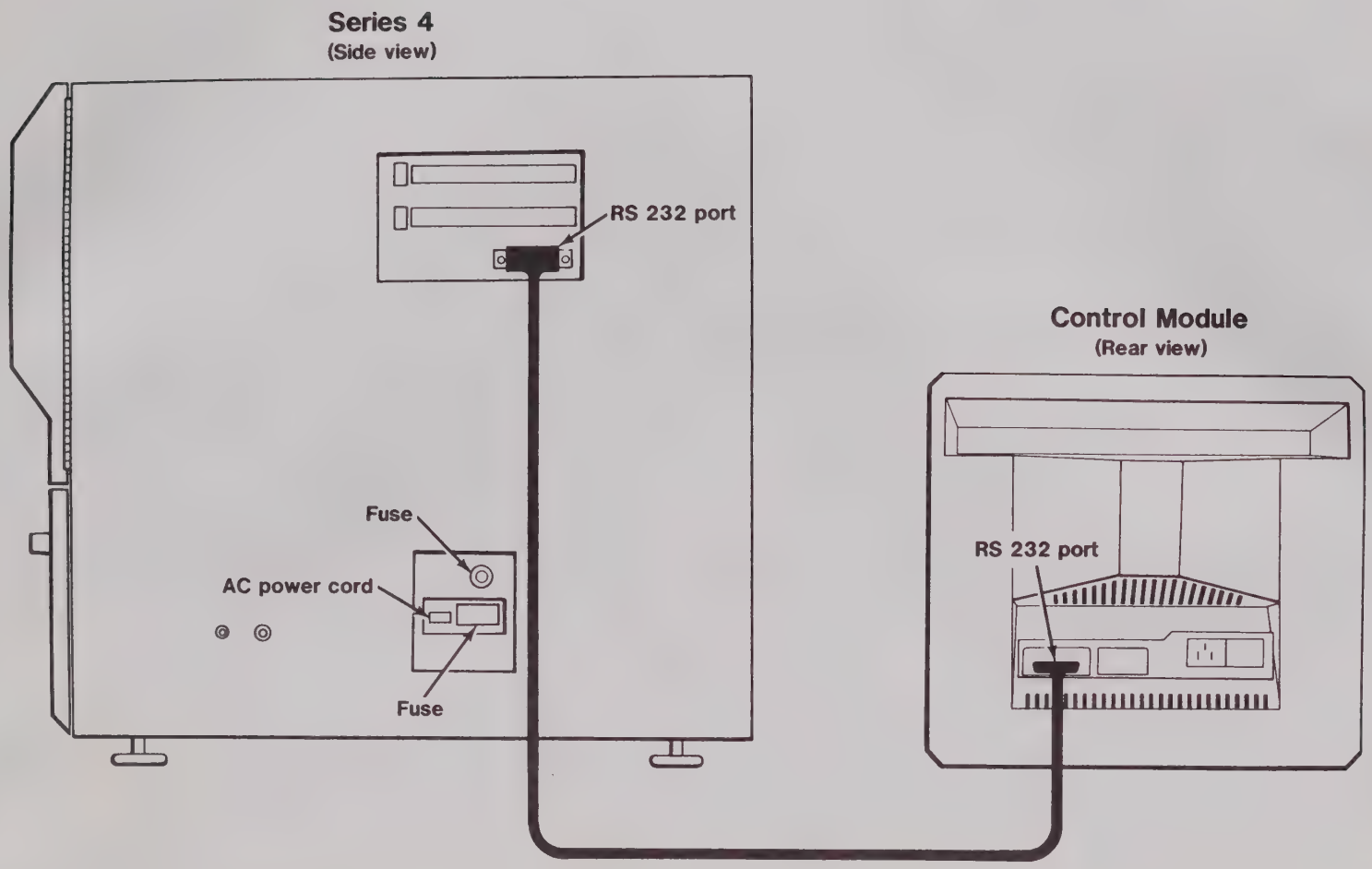


Figure 9-3 - RS-232 Connections Between the Series 4 Pump Module and the Control Module

9.7.3. Configuration

The cable for connecting the Series 4 Pump Module to the Control Module is 2 m (6 feet) long. The Series 4 Pump Module and the Control Module should be placed next to each other with the Control Module on the right. The detector should be placed to the left of the Series 4 Pump Module, and the recorder, if used, to the left of the Detector. The ISS-100 Autosampler is designed to fit on top of the Series 4 Pump Module (see Figure 9-2). The 420B Autosampler Carousel will fit on top of the Series 4 Pump Module, with the controls placed to the right of the Control Module.

Provide a source of regulated (30 to 100 psi) helium for solvent pressurization. The user may supply a solvent vapor vent line to a standard laboratory fume hood or other exhaust if desired.

> **Caution:** DO NOT EXCEED 100 psi.

9.7.4. Electrical Requirements

The Pump Module requires a grounded 117 VAC or 234 VAC source. The Control Module requires a 117 VAC or 234 VAC source. The power requirements for the Series 4 are 600 watts. The electrical requirements for the other instruments to be used with the LC system are listed in separate instruction manuals.

9.8. CONNECTIONS

RS-232 Cable

Connect the RS-232 Cable between the Series 4 Pump Module and the Control Module as shown in Figure 9-3.

9.9. POWER CONNECTION

9.9.1. Voltage Selection & System Fuse

A small printed circuit board located below the system fuse on the left side panel of the Series 4 must be set to correspond to the line voltage available (see Figure 9-4).

To select the proper operating voltage range setting:

(1) Remove the AC line cord from the instrument.

(2) Slide the clear plastic fuse cover all the way to the left.

(3) Rotate the FUSE PULL to the left and remove the fuse.

(4) Pull out the printed circuit board located below the fuse.

(5) Orient the printed circuit board so that the desired voltage setting is legible in accordance with the following:

AC Voltage Range	Legible P.C.B. Setting	Fuse	Part Number
90-110V	100	6.25 amp	0998-1761
105-129V	120	6.25 amp	0998-1761
180-220V	220	4 amp	0998-1616
217-258V	240	4 amp	0998-1616

(6) Push the printed circuit board back into the slot and rotate the FUSE PULL back into the normal position.

(7) Replace the fuse.

(8) Slide the clear plastic fuse cover back to the right.

(9) Replace the power cord.

(10) Check to see that the POWER siwtch on the front of the instrument is OFF.

(11) Connect the line cord to a properly-grounded AC outlet.

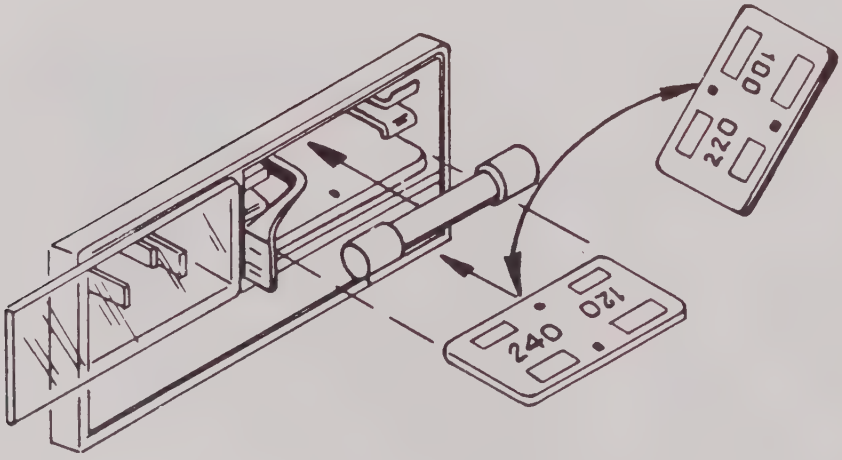


Figure 9-4 - Voltage Selection PC Board

9.9.2. Power Cord

The power cord should be connected to a 20A (117 VAC) or 10A (234 VAC) main power outlet with a good earth ground. The instrument should not be attached to circuits associated with equipment of a heavy-duty nature (e.g., large motors or large or frequent loads (e.g., photo-copying systems, discharge lamps, radio transmitters).

9.9.3. Power Supply Fuse

There are two separate fuses on the Series 4 Pump Module. The system fuse is located next to the power supply cord and is explained above under Voltage Selection. The power supply fuse is in a round fuse holder. This fuse is a 3 amp Slo-Blo fuse for 117 VAC operation (Perkin-Elmer Part No. 0993-1626). For 234 VAC operation, this fuse is a 1.5 amp. Slo-Blo fuse (Perkin-Elmer Part No. 0998-1776).

To replace the fuse:

(1) Turn the power OFF and disconnect the power cord.

(2) Turn the fuse cap counterclockwise and remove the fuse.

(3) Replace the fuse.

If the replacement fuse also blows, contact the Perkin-Elmer Service Department.

9.10. HELIUM SUPPLY

Connect the helium line from a regulated 30-100 psi (maximum) regulated source to the input fitting on the side of the Series 4 Pump Module (see Figure 9-5).

When using a common helium supply for two or more Series 4 instruments, each Series 4 must have its own regulator.

Important: Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before shutting off or disconnecting the helium gas supply.

Important: Relieve the pressure in the Series 4 by opening the door to the solvent pressurization chamber before any period of prolonged shutdown.

Caution: Do not exceed 100 psi.

9.11. OPTIONAL VENT LINE

Connect a vent line (not supplied) from the fitting on the side of the Series 4 Pump Module (see Figure 9-5) to a standard labortory fume hood or other suitable exhaust if desired.

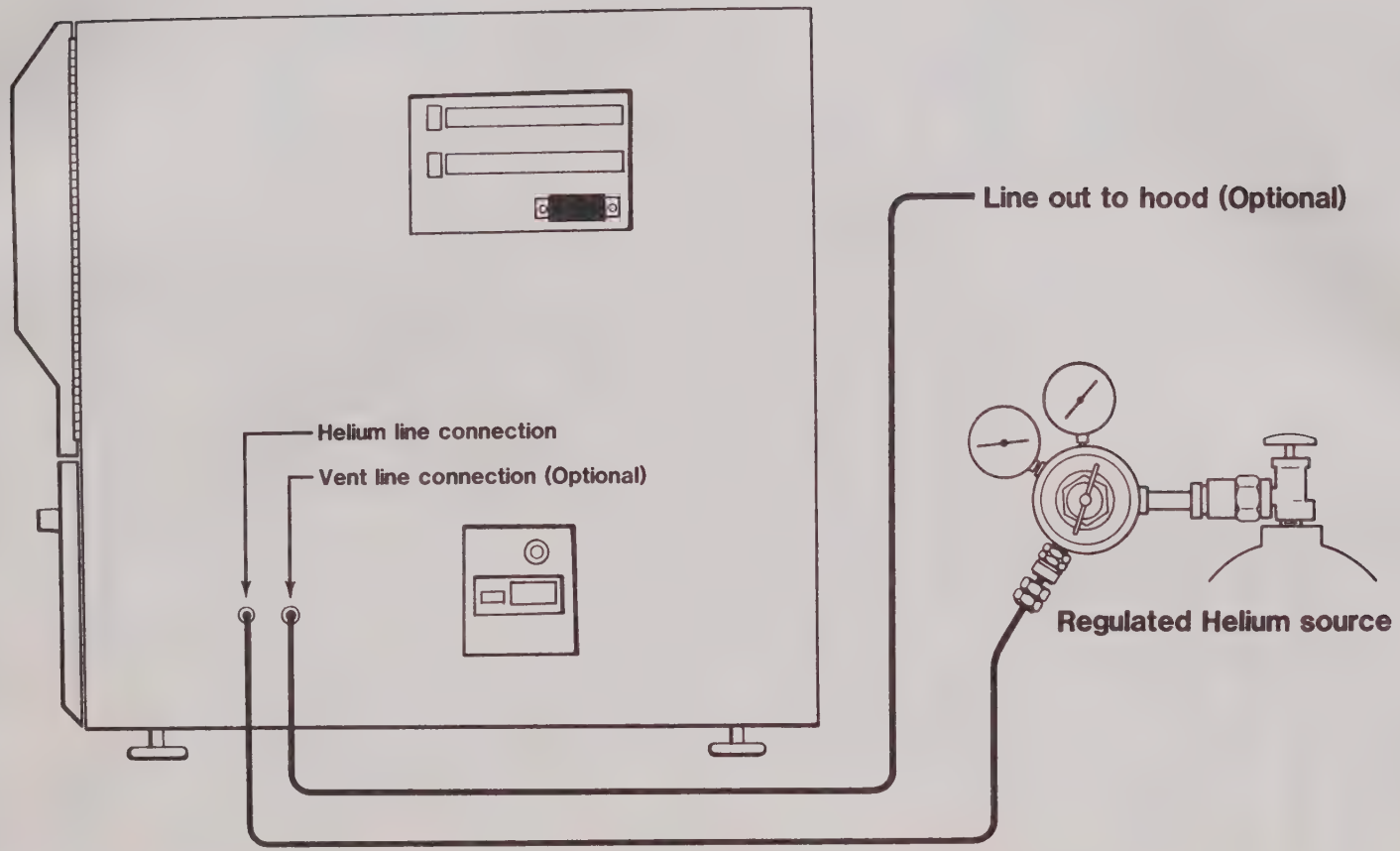


Figure 9-5 - Connections for Helium Supply and Optional Vent Line

9.12. CONNECTIONS TO INJECTOR*

See Figure 9-6 for injector connection.

9.13. CONNECTION TO COLUMN*

See the instructions shipped with the column

9.14. CONNECTIONS TO DETECTOR*

See instructions given in the Detector Manual.

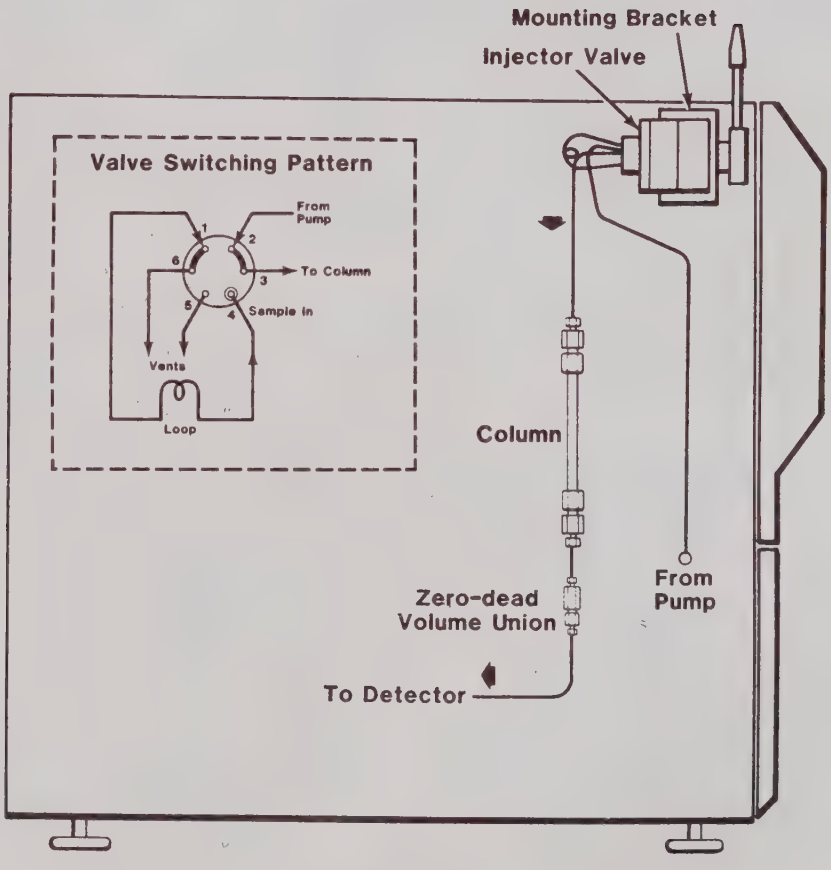


Figure 9-6 - Connection to Injector

*If the Optional Column Oven is to be used, instructions for connection the column to the injector and detector are given in Section 10 of this manual.

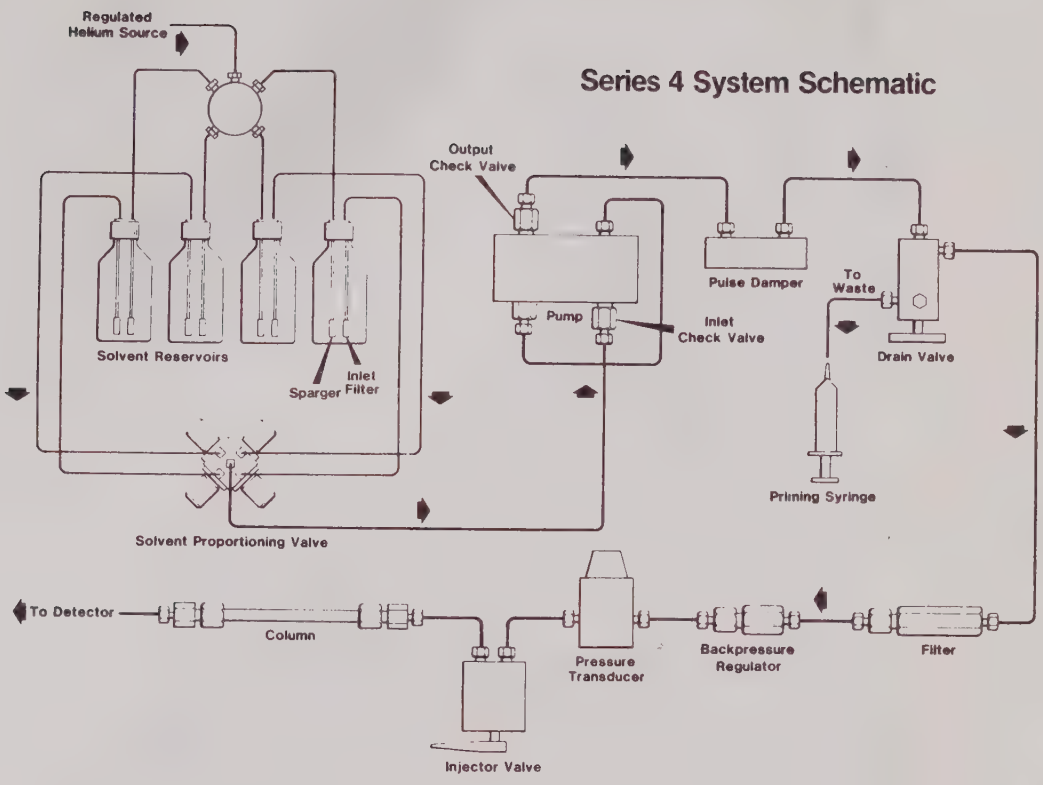


Figure 9-7 - Series 4 Hydraulic Block Diagram

9.15. CONNECTIONS TO THE ISS-100 AUTOSAMPLER

See Figure 9-8, 9-9 and 9-10.

If you are supply your own integrator, use Figure 9-8. If you are using a Perkin-Elmer Sigma 15, use Figure 9-9 with the local interface or Figure 9-10 with the remote interface.

9.16. CONNECTIONS TO THE 420B AUTOSAMPLER

See Figures 9-11, 9-12 and 9-13.

If you are supply your own integrator, use Figure 9-11. If you are using a Perkin-Elmer Sigma 15, use Figure 9-12 with the local interface or Figure 9-13 with the remote interface.

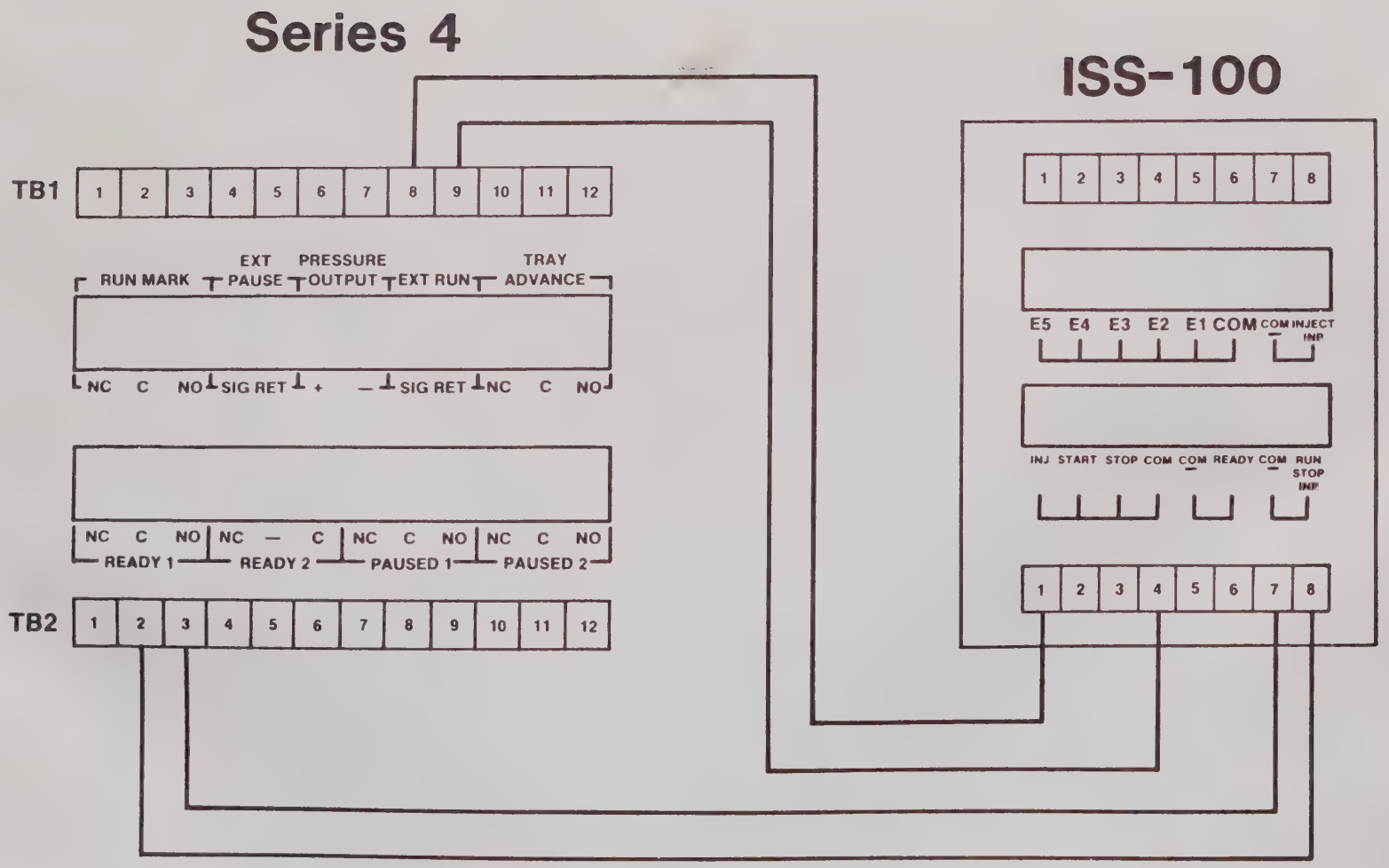

Series 4
ISS-100
TB1
1 2 3 4 5 6 7 8 9 10 11 12
RUN MARK
EXT PAUSE
PRESSURE OUTPUT
EXT RUN
TRAY ADVANCE
NC C NO SIG RET + – SIG RET NC C NO
NC C NO NC – C NC C NO NC C NO
READY 1 READY 2 PAUSED 1 PAUSED 2
TB2
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6 7 8
E5 E4 E3 E2 E1 COM COM INJECT INP
INJ START STOP COM COM READY COM RUN STOP INP
1 2 3 4 5 6 7 8

Figure 9-8 - ISS-100 Autosampler Connnection

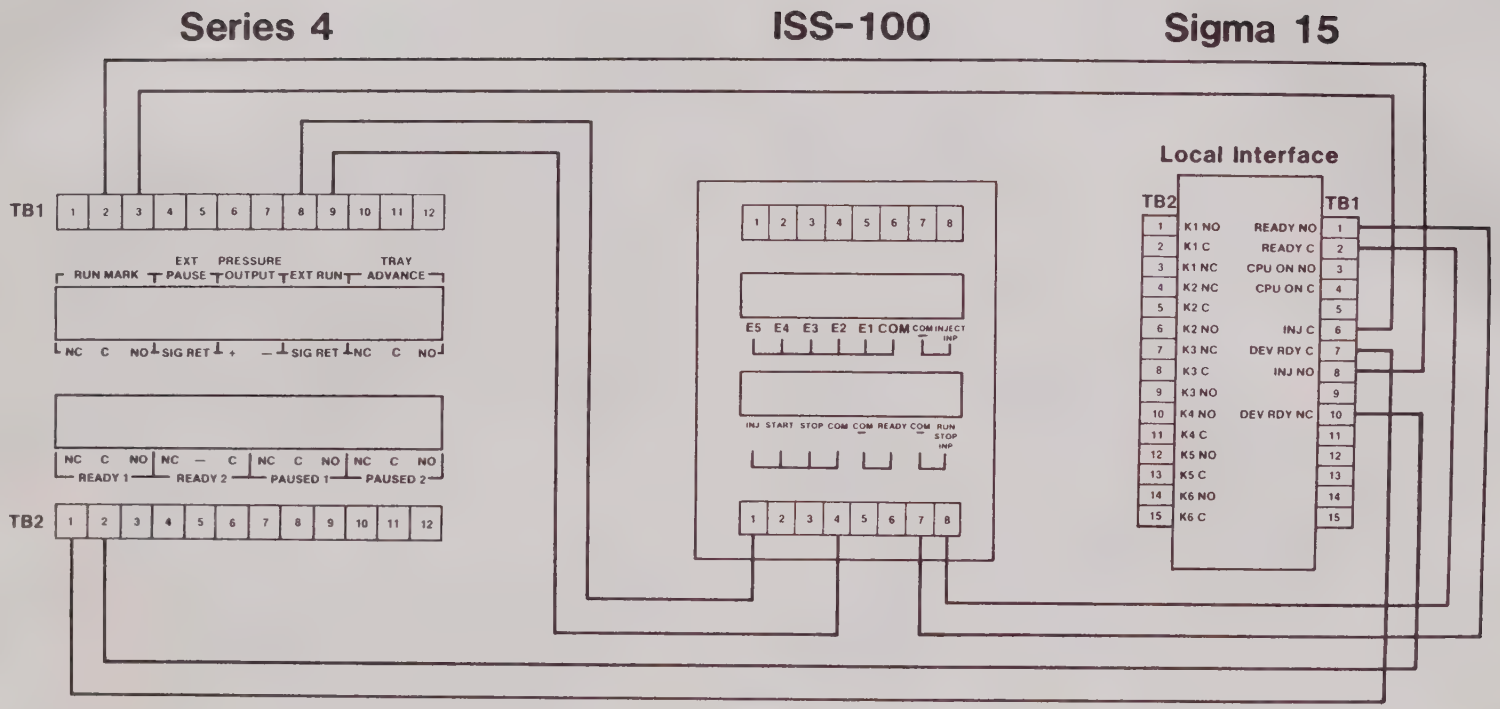


Figure 9-9 - ISS-100 Autosampler Connection to Sigma 15 - Local Interface

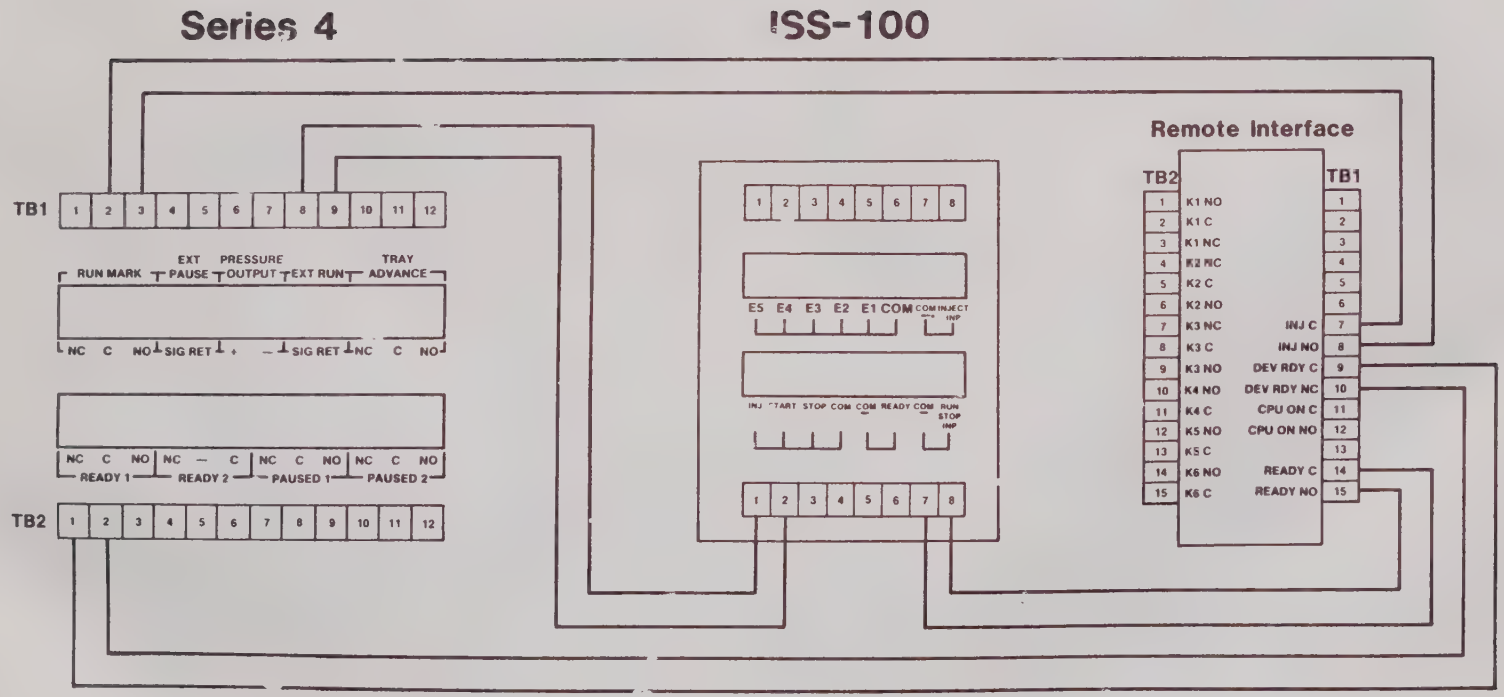


Figure 9-10 - ISS-100 Autosampler Connection to Sigma 15 - Remote Interface

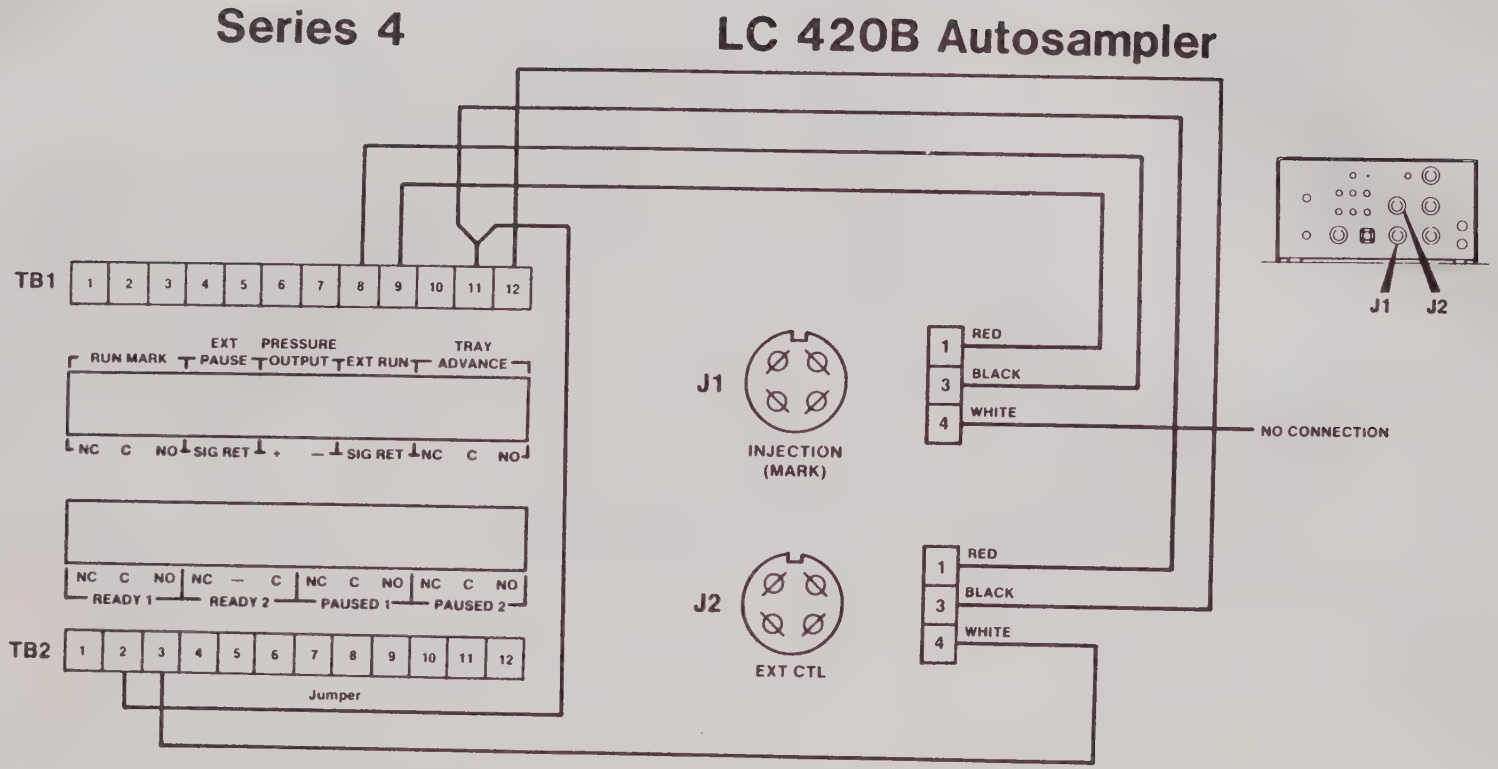


Figure 9-11 - 420B Autosampler Connection

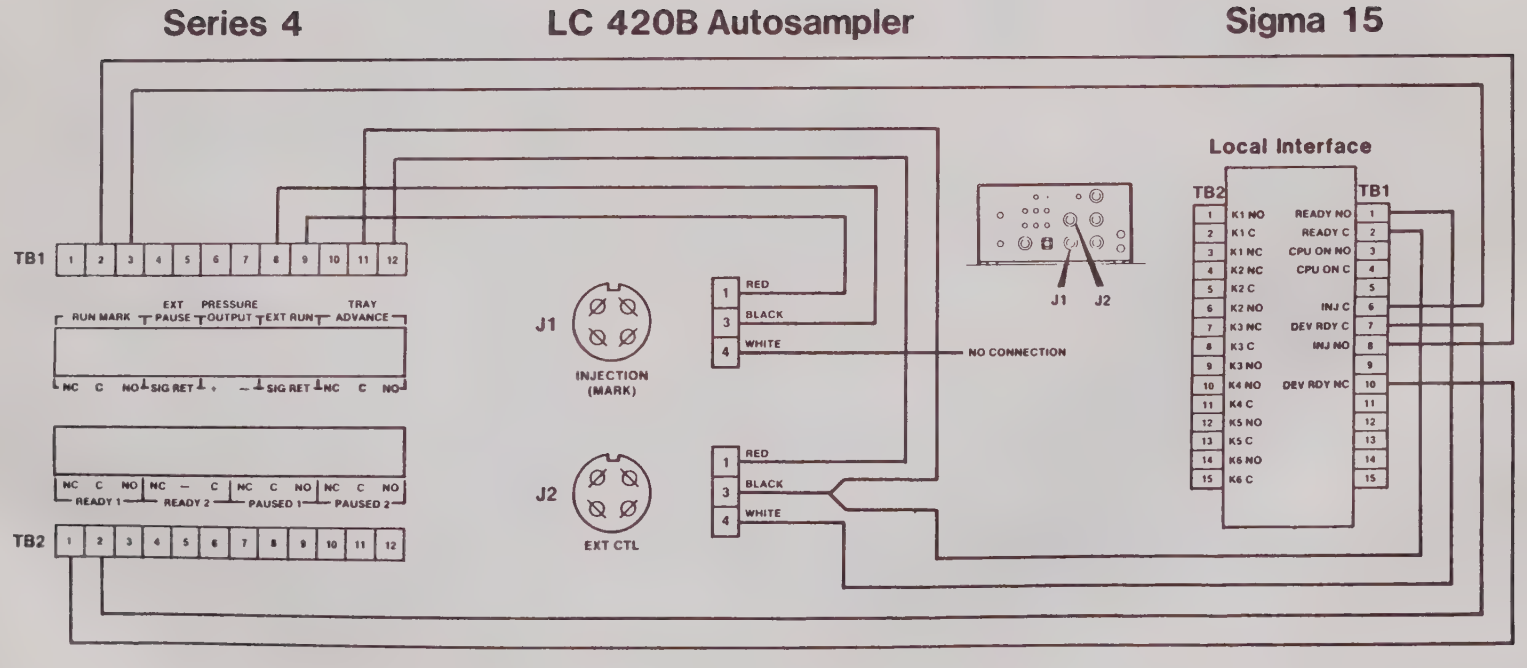


Figure 9-12 - 420B Autosampler Connection to Sigma 15 - Local Interface

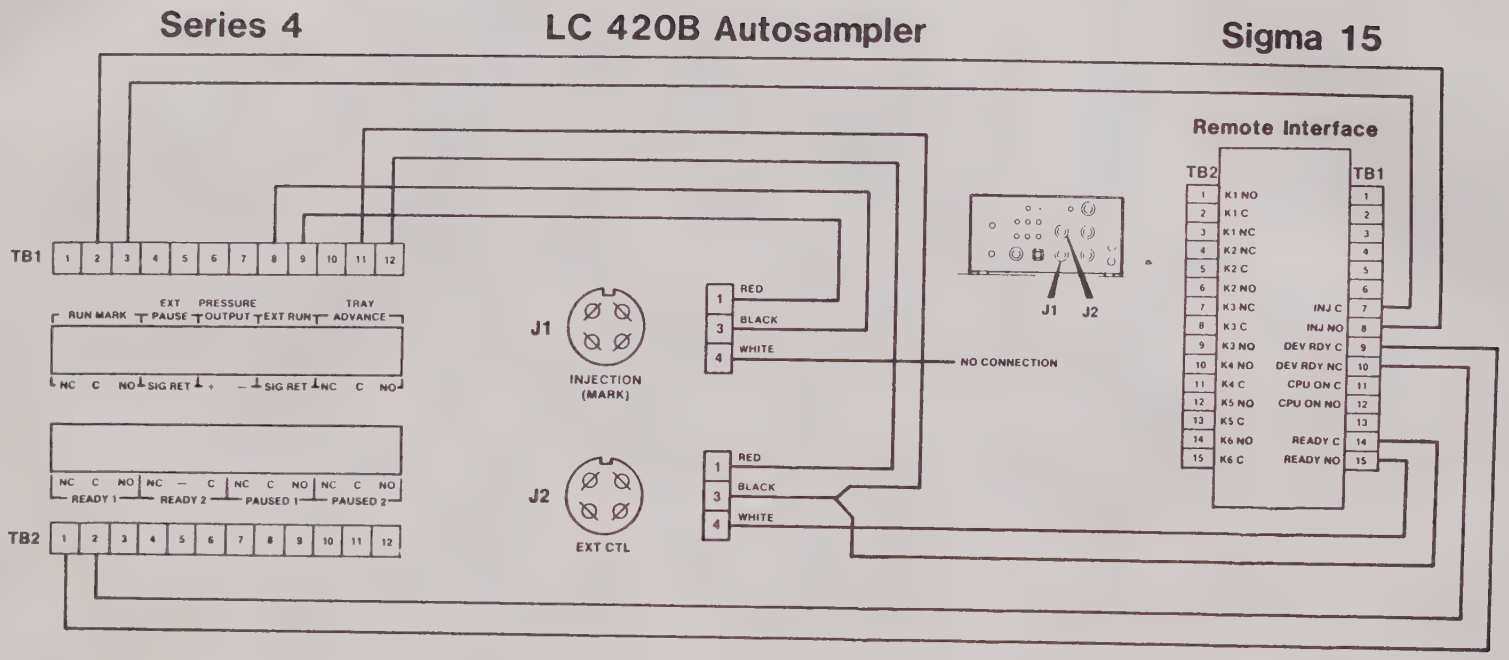


Figure 9-13 - 420B Autosampler Connection to Sigma 15 - Remote Interface

9.17. ADJUSTING THE PULSE DAMPER SEALING SCREW

The Pulse Damper Sealing Screw is set in the low pressure, or "out" position for shipping purposes. While the sealing screw is in this position:

1. No pressure should be applied to the LC system ports.

2. The LC system ports cannot be isolated from atmosphere (i.e., all system ports plugged, or valve closed).

3. The temperature must not exceed 60° to 65°C maximum.

9.17.1. Installation

The Pulse Damper Sealing Screw must be set in the high pressure, or "in" position at installation to assure proper functioning of the pulse damper. Perform the adjustment as follows:

(1) Ensure that the temperature of the pulse damper is normal laboratory ambient temperature (40°C, 104°F maximum).

> **Caution:** Extensive damage can result if the Pulse Damper Sealing Screw is adjusted while the Series 4 Pump Module is warm from shipping or storage.

(2) Check that the system ports are open to atmosphere to prevent fluid fill from exceeding the low pressure limitation.

(3) Open the drain by turning the valve counterclockwise.

(4) Using a 1/4-in. Allen wrench, turn the sealing screw clockwise until it reaches the bottom of its adjustment.

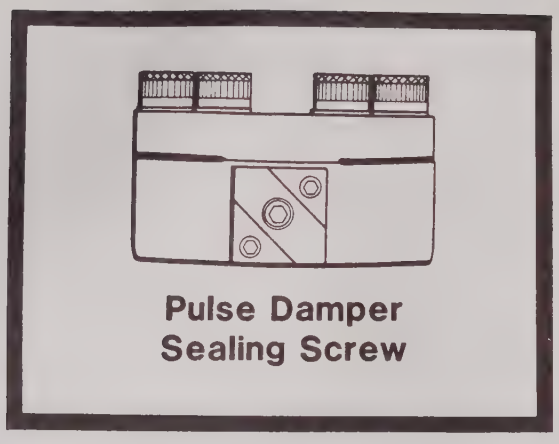
When the sealing screw is in the full "in" position, the high pressure seal is achieved. In this position, the upper temperature limit is 40°C, 104°F (nominal).

9.17.2. Storage or Reshipment

If the Series 4 Pump Module is to be stored for a lengthy period of time, or if it must be reshipped, the Pulse Damper Sealing Screw should be returned to the low pressure, or "out" position.

(1) Ensure that the system ports are open to atmosphere to prevent unnecessary vacuum condition in the pulse damper.

(2) Using a 1/4 in Allen wrench, loosen the Pulse Damper Sealing Screw by turning the wrench counterclockwise until the outer stop is contacted.



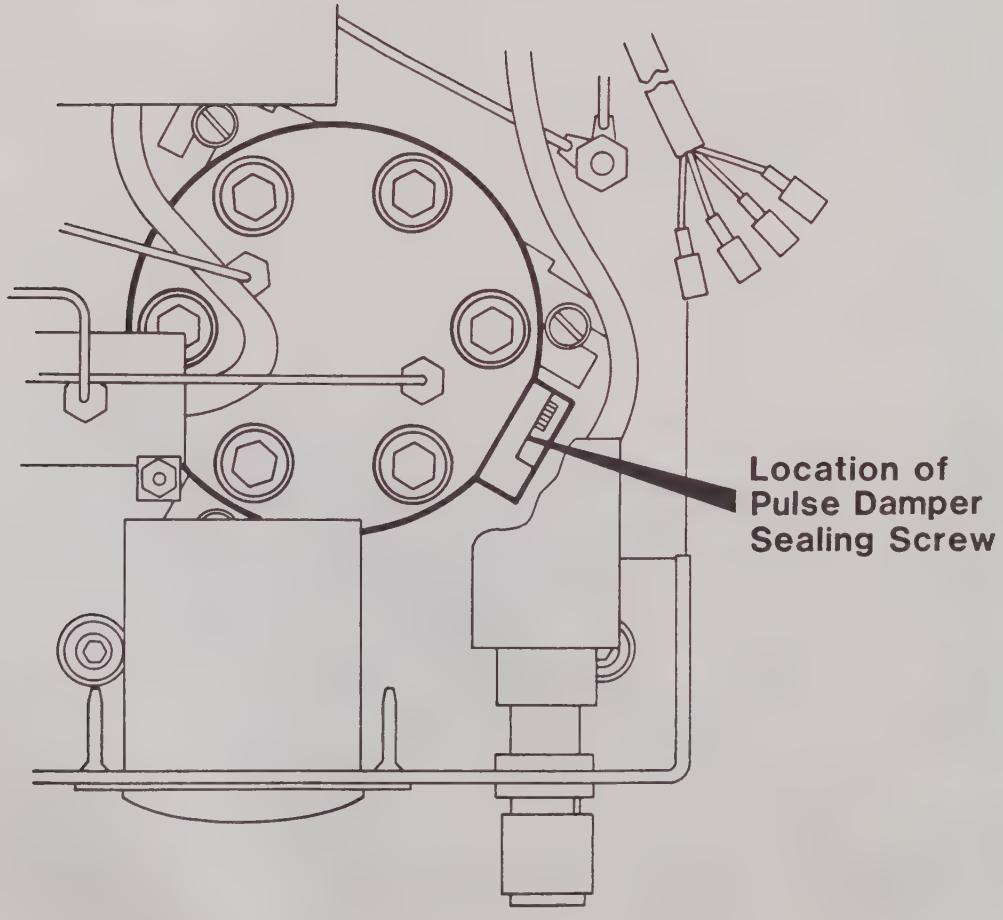


Figure 9-14 - Pulse Damper Sealing Screw

9.18. SET-UP OF SERIES 4 PUMP MODULE

The Series 4 Pump Module is shipped completely assembled. It is necessary to place the solvent reservoirs in the solvent compartment and to make the appropriate connections and adjustments previously described.

> **CAUTION:** Make sure that the Pulse Damper Sealing Screw has been placed in the Operating Position before proceeding with installation.

(1) Rotate the pressure regulator knob counterclockwise to close the valve.

(2) Unpack the solvent reservoirs.

(3) Fill each reservoir with an appropriate solvent. Do not fill beyond the 1000 ml mark. Notice that the solvent lines are labeled A, B, C and D.

(4) Cap each reservoir, checking to see that the solvent input filters rest on the bottoms of the reservoirs. Hold the cap while turning the reservoir to avoid the twisting lines.

(5) Reservoirs which will not be used may be removed and the lines capped. The cap for the helium input line is P/N 0254-2416. The cap for the vent line is 0254-2415.

(6) Place the reservoirs in the pressurization chamber and latch the door.

(7) Check to see that the AUTO RUN/MAN RUN switch on the right side of the Series 4 Pump Module is in the MAN RUN position (or in the appropriate position).

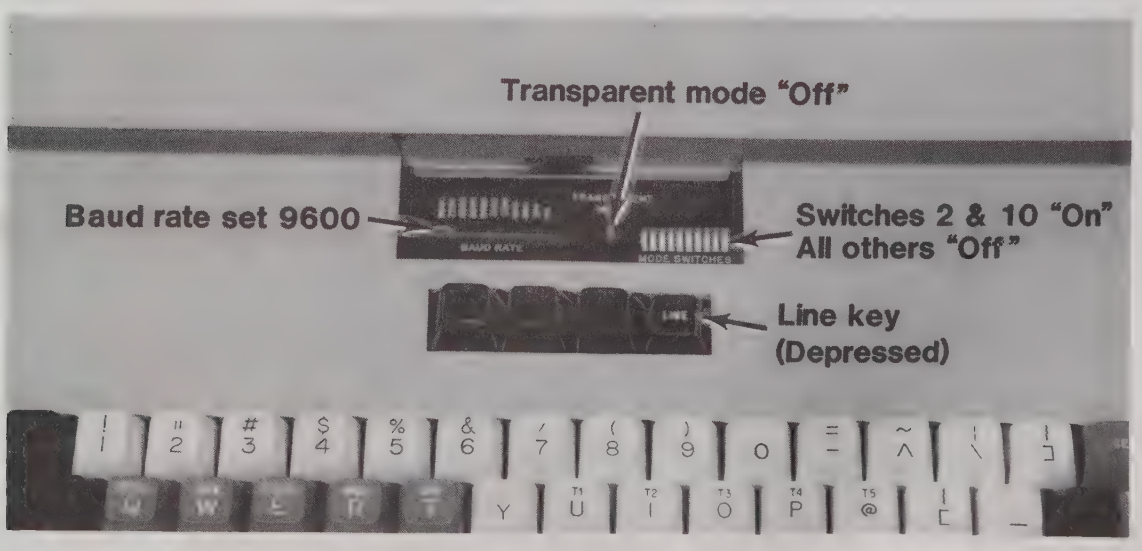


Figure 9-15 - Data Line Set-Up

9.19. SET-UP OF THE CONTROL MODULE

The Control Module is shipped ready for operation. It is advisable, however, to check to see that the Data Line switches are set properly and to make sure that the LINE key is depressed (see Figure (9-15).

9.19.1. Data Line Switches

The Data Line switches should be set as follows:

Baud Rate: 9600

Transparent Mode: OFF

Parity Switches (Even Parity)

1 OFF
2 ON

Mode Switches

3 OFF
4 OFF
5 OFF
6 OFF
7 OFF
8 OFF
9 OFF
10 ON

Turn the Control Module on (see Fig. 9-16) and adjust the intensity control for the desired brightness of the screen display.

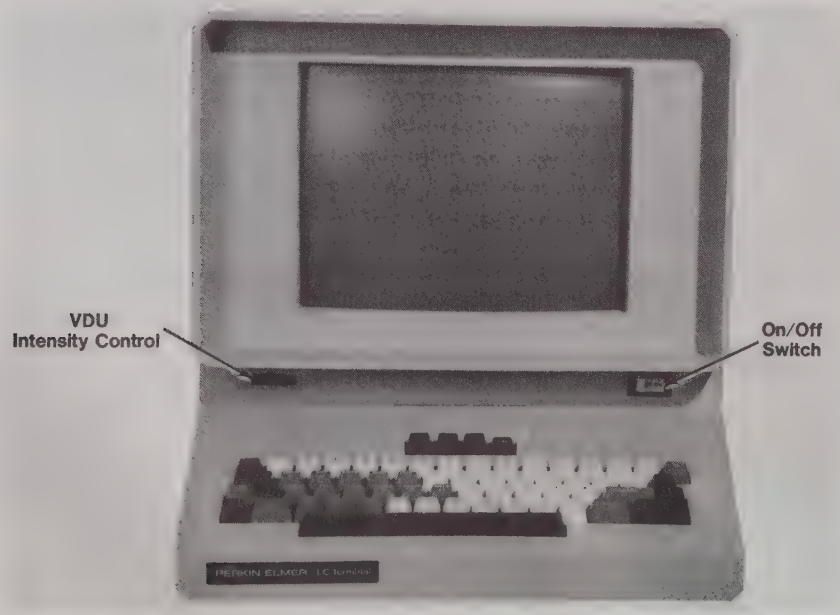


Figure 9-16 - Control Module ON/OFF Switch and Intensity Control

9.19.2. Setting the Parity and the Baud Rate on the Series 4 Pump Module

The Parity of the Series 4 is determined by a suitcase jumper on SP2 on the MPU board of the Series 4 Pump Module. For even parity, the jumper should be on AB. For odd parity, the jumper should be on BC. If the parity is set incorrectly, pull the jumper straight out off the pins, and carefully push onto the correct pair of pins.

The baud rate of the Series 4 is determined by a suitcase jumper on SW1 on the MPU board of the Series 4 Pump Module. To change the baud rate, carefully pull the suitcase jumper straight out off the pins, and carefully push onto the correct pair of pins. The available baud rates and the appropriate pin pairs are marked on the board.

To access the MPU board, remove the 4 screws which hold the back panel on the instrument. Look for the blue suitcase jumpers. You will see the baud rate pins near the upper left-hand corner. The parity pins are on the left side of the board about half way down.

9.20. DEGASSING (SPARGING) THE SOLVENTS

Note: Do not fill the reservoirs beyond the 1000 ml line.

(1) With the door to the solvent chamber OPEN, rotate the pressure regulator knob clockwise to start the helium.

(2) Sparge (bubble) the solvent(s) for approximately 5 minutes.

> **Note:** Sparging is most effective when all the reservoirs are filled to approximately the same level.

9.21. PRESSURIZING THE SOLVENTS

(1) Close the door to the solvent chamber and turn the knob to latch it. The reservoirs will then automatically pressurize. The bubbles will slow and eventually stop forming.

> **Warning:** Whenever the door to the solvent chamber is opened, the solvent reservoirs depressurize automatically. This is a safety feature. Do not attempt to defeat the door interlock.

(2) Adjust the pressure by rotating the pressure regulator knob clockwise until the pressure gauge reads 7 to 9 psi.

> **Note:** At pressures above 10 psi the release valve opens.

9.22. PRIMING THE PUMP AND PURGING THE SYSTEM

Note: A Method must have been generated and must be displayed on the screen in order for the pump to run.

(1) Open the cover to the pump compartment. (The cover to the pump compartment is hinged on the bottom.)

(2) Connect the priming syringe to the flush port.

(3) On the Control Module press the blue RESET key and hold it down while you press the PURGE key. The prompt line will say:

RESERVOIR?

(4) Type in the letter A. The prompt line on the VDU Screen will say:

PURGE FLOW?

Note: If you type in anything other than the letters A, B, C or D, the LC Terminal will beep and the prompt:

ERROR, CE

will appear. Type in the correct letter and press the ENTER key.

(5) To get a flow rate of 0 (zero), press the ENTER key*. The valve will audibly click open.

(6) Go back to the Pump Module. Open the drain valve by turning the handle counterclockwise.

(7) Collect about 20 milliliters of solvent in the syringe or continue until air bubbles have stopped coming through.

(8) Stop the flow into the syringe by turning the drain valve handle clockwise. Make sure that the valve is fully closed before removing the priming syringe.

(9) Press the blue RESET key and hold it down while you press the STOP key. This will exit from the PURGE mode.

(10) Remove the priming syringe and dispose of the solvent in an appropriate waste container.

*Note: A purge flow rate is used when the column is to be purged. Choose a purge flow rate that is appropriate for the backpressure of the column.

10 OPTIONAL COLUMN OVEN

Section		Page
10.1	Column Oven Installation	10-1
10.2	Unpacking	10-1
10.3	Parts Supplied	10-1
10.4	Connection to Series 4 Pump Module	10-1
10.4.1	Mechanical Connection	10-1
10.4.2	Electrical Set-Up	10-1
10.4.3	AC Connection	10-1
10.5	Installing the Column Connections to Injector and Detector	10-1
10.6	Changing Column Oven Inserts	10-3
10.7	Operation	10-5
10.7.1	Timed Method Operation	10-5
10.7.2	Untimed Isocratic Method Operation	10-5
10.7.3	Chain Operation	10-5
10.8	Solvents with Auto-Ignition Temperatures Below 110°C	10-7
10.9	Maintenance	10-7
10.10	Service	10-8
		10-8

10. OPTIONAL COLUMN OVEN

10.1. COLUMN OVEN INSTALLATION

Installation of the Series 4 Optional Column Oven is to be performed only by a Perkin-Elmer Service Engineer.

10.2. UNPACKING

The Column Oven is shipped in one box, completely assembled except for the ribbon cable connector. When unpacking, check for signs of shipping damage. Report any damage to the carrier and to the Perkin-Elmer Corporation immediately.

10.3. PARTS SUPPLIED

The Column Oven (Perkin-Elmer Part No. 0254-0196) is shipped with standard column inserts designed to accept standard analytical columns in common use (12, 15, 25, or 30 cm long x 1/4" OD). Optional inserts are available for other frequently-used columns. Contact your local Perkin-Elmer representative for further information.

10.4. CONNECTION TO SERIES 4 PUMP MODULE

10.4.1. Mechanical Connection

Remove the left-hand panel from the Series 4 Pump Module. This panel is held in place with 6 screws, 4 on the front of the Pump Module and 2 on the rear.

These same 6 screws are used to attach the Oven to the Series 4 Pump Module.

10.4.2. Electrical Set-Up

Check to see that the jumper wires on TB4 (see Figure 10-1) are properly connected as follows:

For 230 VAC operation, jump terminal 5 to terminal 6 only.

For 117 VAC operation, jump terminal 4 to terminal 5 and jump terminal 6 to terminal 7.

10.4.3. AC Connection

The 2 Fast-On connectors and the chassis ground ring lug from the Oven should be connected to the TB5 barrier strip located next to the Series 4 Power Supply (see Figure 10-2). The BLUE wire connects to position 4 on TB5 and the GREY wire connects to position 3 on TB5. The chassis ground ring lug should be connected to the E5 ground stud.

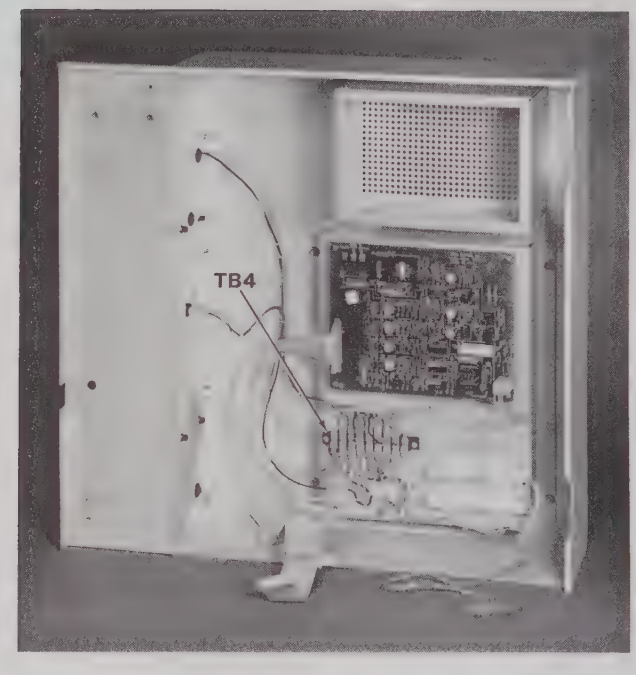


Figure 10-1 - Column Oven TB4

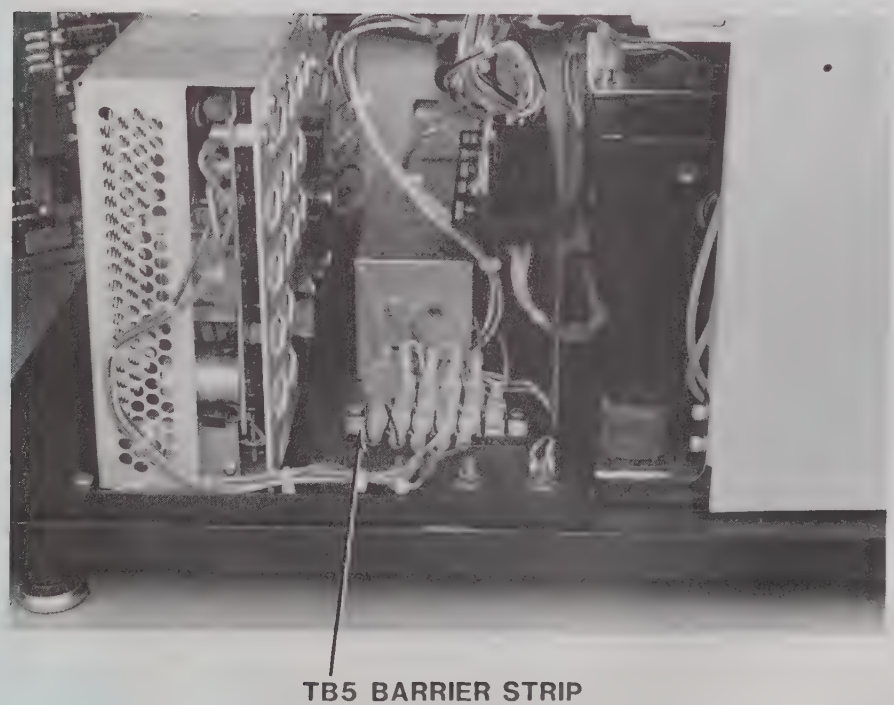


Figure 10-2 - TB5 Barrier Strip

Connect one end of the ribbon connector cable to J18 on the Oven Module. Connect the other end to J10 on the MPU board and snake it behind the I/O Board and across to the Oven Module. (See Figure 10-3.)

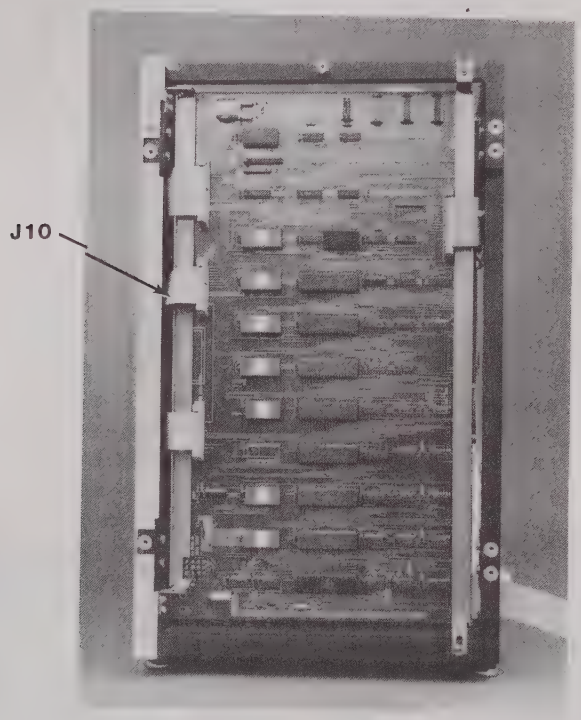


Figure 10-3 - Connector J-10

10.5. INSTALLING THE COLUMN: CONNECTIONS TO INJECTOR AND DETECTOR

Connect the column to appropriate lengths of injector and detector tubing.

Open the oven door and place the column in the oven cavity.

Route the detector tubing out through the exit hole in the inner oven housing which is located in the hinge, and through the round hole in the left side of the outer oven housing (see Figure 10-4).

Support the column with the input tubing and hold it in place in the cavity while closing the door.

This will support the column firmly while you connect the tubing to the injector and to the detector.

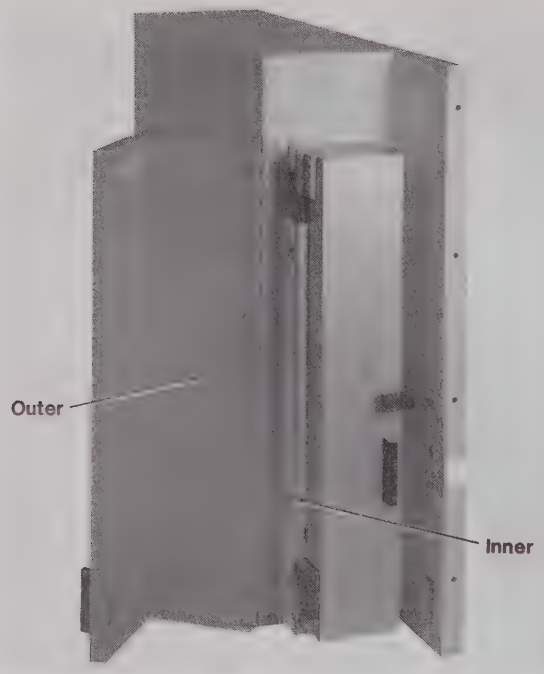


Figure 10-4 - Detector Tubing Exit

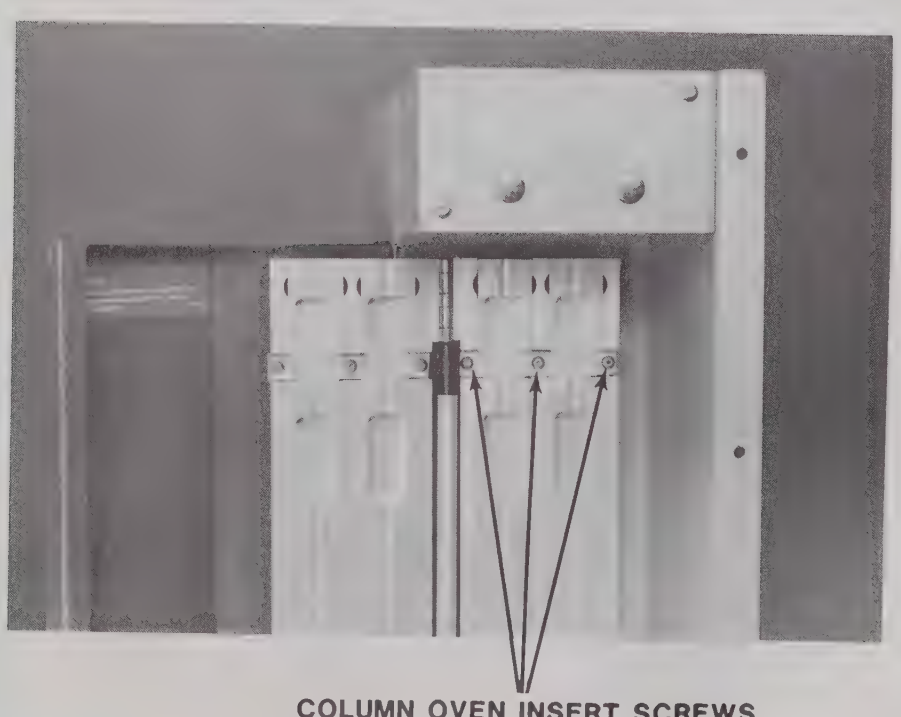


Figure 10-5 - Changing Column Oven Inserts

10.6. CHANGING COLUMN OVEN INSERTS

Warning: Make sure that the oven has cooled down to ambient temperature before attempting to touch column oven inserts.

There are two "clamshell" column inserts on each oven panel. Six screws and retaining clips are used to hold the 2 inserts on each panel; the two center retaining clips hold both column inserts.

To remove the column inserts, remove the 6 screws and retaining clips. (See Figure 10-5. Since springs behind the heating bar hold it in contact with the oven inserts, when the screws and retaining clips are removed, the inserts will pop out easily.

To insert the new column insert, hold it in place and replace the screws and retaining clips. Note that the clips with the hole in the center should be used in the center.

10.7. OPERATION

Control of the column oven temperature is from the Control Module keyboard.

When the column oven is connected, the TEMPERATURE parameter will be requested when the Series 4 Method is generated.

If this entry is defaulted in the Method, the temperature control will be OFF.

If the oven is to be used, a value between 35^0C and 99^oC should be entered in the Method. If the value entered is less than 35^oC, the temperature control will default to an oven power OFF condition.

10.7.1. Timed Method Operation

When a timed run is programmed with a heated oven, then the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. When the cool-down sequence is complete, the Series 4 pump will shut down.

CAUTION: The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

10.7.2. Untimed Isocratic Method Operation

If a heated oven is to be used with an untimed isocratic run, there is no automatic cool-down sequnece to protect the column. The operator must modify the Method without stopping the pumps to turn off the oven and then must allow the solvent to continue to flow for a sufficient time to cool down the column.

To provide a cool-down sequence to protect the column after running in the untimed isocratic mode:

(1) Press the ENTER key and hold it down while pressing the EQUIL key. This will step to the equilibration sector.

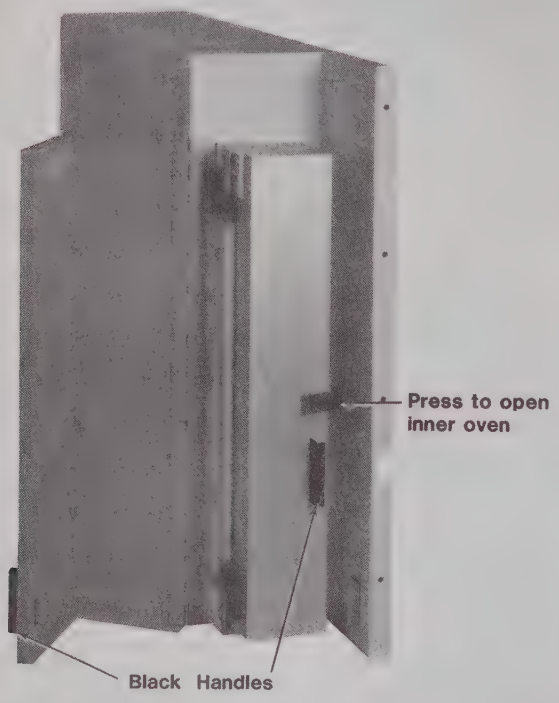
CAUTION: DO NOT STEP TO THE SHUTDOWN SECTOR TO MODIFY THE TEMPERATURE.

(2) Press the RESET key and hold it down while pressing the MODIFY key.

(3) Press the TEMP parameter key to step directly to the temperature parameter.

(4) Type 10 and press the enter key. This will enter 10°C, which will turn the oven OFF.

(5) Allow the solvent to flow for a sufficient period of time to cool the column before using the STOP key to stop the pump.



Warning: The surface of the column oven is hot. Use the black handles only to open the oven door. Allow sufficient time for the oven to cool down before attempting to open it. the inner oven door latch should be pressed to open. (See Figure 10-6).

Figure 10-6 - Column Oven Door Handles

10.7.3 Chain Operation

When chaning together several methods which use the OVEN, the Methods should be arranged in the Chain so that the temperature increases as the Chain progresses. All Methods which do not use the OVEN (temperatures below 35 °C) should come before Methods which use temperature control.

When a timed run is programmed with a heated oven, when the Ready Time runs out, the Series 4 will automatically enter a cool-down sequence in which the oven will turn off and the solvent will continue to flow until the oven is cool. when the cool-down sequence is complete, the Series 4 pump will shut down.

> **CAUTION:** The cool-down sequence protects the column. Do not accidentally use the STOP key to stop the pump during the cool-down sequence.

10.8. SOLVENTS WITH AUTO-IGNITION TEMPERATURES BELOW 110 °C

Certain solvents have a temperature at which they are combustible upon contact with a heated surface even in the absence of a spark or other source of ignition. For your convenience a list of common solvents with their auto-ignition temperatures is given below.

> **Warning:** DO NOT USE CARBON DISULFIDE OR OTHER SOLVENTS WITH AN AUTO-IGNITION TEMPERATURE BELOW 110 °C.

AUTO-IGNITION TEMPERATURES OF COMMON LC SOLVENTS

SOLVENT	AUTO-IGNITION °C
pentane	309
hexane	261
isoctane	418
petroleum either (naptha)	288
cyclohexane	260
i-propyl ether	443
diethyl ether	180
benzene	562
THF	321
methylethylketone	474
acetone	538
acetonitrile	524
ethanol	423
methanol	446
propanol	404
carbon disulfide	100
dioxane	366
dimethylformamide	445
toluene	536
ethyl acetate	524
isopropanol	455

10.9. MAINTENANCE

There is no routine maintenance to be performed on the Series 4 Column Oven other than wiping up spills that could corrode the aluminum surfaces.

10.10. SERVICE

Service on the Series 4 Column Oven should be performed only by a Perkin-Elmer Service Engineer.

11 SPECIFICATIONS

Section		Page
11.1	Instrument Type	11-1
11.2	Principles of Operation	11-1
11.3	System Configuration	11-1
11.4	Numerical Specifications: Series 4 Quanternary Solvent Delivery System	11-1
11.4.1	Flow Rates	11-1
11.4.2	Flow Rate Indication	11-1
11.4.3	Flow Rate Accuracy	11-1
11.4.4	Flow Rate Reproducibility	11-1
11.4.5	Maximum Pressure	11-1
11.4.6	Pressure Sensor	11-1
11.4.7.	Pressure Limits	11-2
11.4.8	Pressure Indication	11-2
11.4.9	Pressure Pulsation	11-2
11.4.10	Solvent Compressibility	11-2
11.4.11	Piston Displacement	11-2
11.4.12	Compositional Accuracy	11-2
11.4.13	Compositional Precision	11-2
11.4.14	Compositional Resolutions	11-2
11.4.15	Gradient Compositional Accuracy and Reproducibility	11-2
11.4.16	Solvent Storage	11-2
11.4.17	Pump Materials	11-2
11.4.18	Programmable Methods	11-2
11.4.19	Programmable Method Chaining	11-2
11.4.20	Safe Memory	11-2
11.4.21	Video Display Unit	11-3
11.4.22	Keyboard	11-3
11.4.23	Visual Display	11-3
11.4.24	Column Oven (Optional)	11-3
11.5	External Services	11-3
11.5.1	Electrical Requirements	11-3
11.5.2	Laboratory Type	11-3
11.5.3	Operator Skill	11-3
11.6	Dimensions and Weight	11-3
11.6.1	Series 4 Pump Module	11-3
11.6.2	Series 4 Pump Module with Optional Oven	11-4
11.6.3	Control Module	11-4
11.7	Configuration	11-4
11.8	Options	11-4
11.8.1	Standard Configuration	11-4
11.9	Installation	11-4

11 SPECIFICATIONS

11.1 INSTRUMENT TYPE

The Series 4 is a microcomputer-controlled, single piston rapid-refill pump with four switchable inlet valves, which provides solvent blending for isocratic and multi-segment linear and non-linear gradients suitable for Liquid Chromatography.

11.2 PRINCIPLES OF OPERATION

The basic instrument is a pumping system consisting of one pump, with microcomputer-controlled flow rate. The composition of the mobile phase is selected by means of a microcomputer-controlled 4 solvent proportioning valve. When the pump is used to pump different solvents, the composition of the mixed mobile phse can be constant (isocratic), or varied with time (gradient) and/or the flow rate can be varied with time. Two curves can be generated simultaneously. For example, if the flow rate varies, then one solvent curve can be run. If the flow rate is held constant, then two solvent curves can be run, a third solvent can be held constant and the fourth is the balance or complement to 100%.

11.3 SYSTEM CONFIGURATION

The basic Series 4 instrument with column and injector valve, when connected to an LC 75/85 or other stand-alone detector, will provide a complete chromatographic system. The Series 4, in conjunction with the LC 75/85 Autocontrol, Autosampler and an automatic Stop-Flow Valve, will provide a chromatographic system with additional features such as unattended operation and on-line spectroscopic analysis of peaks. With the addition of a Sigma Console or Chromatography Data Station, quantitative results can be computed and reported.

11.4 NUMERICAL SPECIFICATIONS: SERIES 4 QUATERNARY SOLVENT DELIVERY SYSTEM

11.4.1. Flow Rates:	0.1 to 9.9 mL/min in 0.1 mL/min increments
11.4.2. Flow Rate Indication:	VDU
11.4.3. Flow Rate Accuracy:	±1% or better at 1 mL/min H_2O and 6.9 MPa (1000 psi)
11.4.4. Flow Rate Reproducibility:	±0.3% (coefficient of variation) or better at 1 mL/min H_2O and 6.9 MPa (1000 psi)
11.4.5. Maximum Pressure:	42 MPa (6200 psi) at all flows
11.4.6. Pressure Sensor:	Flow-through pressure transducer

11.4.7. Pressure Limits:	User selectable upper and lower limits for each method
11.4.8. Pressure Indication:	VDU display of set limits and actual
11.4.9. Pressure Pulsation:	Maximum of 2% amplitude at 1 mL/min H_2O and 6.9 MPa (1000 psi)
11.4.10. Solvent Compressibility:	Automatic microprocessor compensation
11.4.11. Piston Displacement:	100 uL
11.4.12. Compositional Accuracy	Typically equal to or better than ±0.5 absolute (with 2σ range of ±0.5%)
11.4.13. Compositional Precision:	Typically ±0.1% absolute or better over entire working range
11.4.14. Compositional Resolutions:	Settable in 0.1% increments over entire working range
11.4.15. Gradient Compositional Accuracy and Reproducibility:	Typically ±1% absolute or better
11.4.16. Solvent Storage:	Enclosed compartment for four standard solvent bottles with solvent degassing and pressurization (standard)
11.4.17. Pump Materials:	All materials in contact with solvents are type 316 stainless steel, glass, sapphire or fluorocarbon polymer
11.4.18. Programmable Methods:	10 (9 in storage plus current method)
11.4.19. Programmable Method Chaining:	The stored methods can be programmed into a multiple method chain of up to 100 components
11.4.20. Safe Memory:	Battery back-up for at least 4 hours for all stored data

11.4.21. Video Display Unit: 29 cm/12 in (diagonal measurement) CRT

11.4.22. Keyboard: Full alphanumeric keyboard with dedicated command keys

11.4.23. Visual Display: All selected method parameters displayed and current pump status on single screen page

11.4.24. Column Oven (Optional):

1.	Temperature range	35° to 99°C
2.	Temperature settability	1°C
3.	Temperature accuracy	±1°C
4.	Temperature stability	Typically ±0.1°C

11.5. EXTERNAL SERVICES

11.5.1. Electrical Requirements:

Voltage
100 - 125V or 100 - 240V
Frequency
50/60 Hz
Power Requirements
200 watts, 600 watts with optional oven

11.5.2. Laboratory Type: Typical organic chemistry laboratory environment

11.5.3. Operator Skill: Typical chemist or laboratory technican level of skill

11.6. DIMENSIONS AND WEIGHT

11.6.1. Series 4 Pump Module

Height: 54 cm (21.25 in.)
Width: 32 cm (12.5 in.)
Depth: 58 cm (23 in.)
Weight: 28.4 kg. (62.5 lbs.)

11.6.2. Series 4 Pump Module with Optional Oven

Height: 54 cm (21.25 in.)
Width: 45 cm (17.5 in.)
Depth: 58 cm (23 in.)
Weight: 37 kg. (82 lbs.)

11.6.3. Control Module

Height: 36 cm (14 in.)
Width: 38 cm (15 in.)
Depth: 55 cm (21.5 in.)
Weight: 14 kg. (30 lbs.)

11.7. CONFIGURATION

The basic system consists of two modules: The Series 4 Solvent Delivery Module and the Control Module, coupled by a six-foot RS-232 cable. The basic Series 4 includes four reservoirs, gradient forming rapid-refill pump and related electronics. It is capable of delivering solvent mixtures to an injector and column selected from the Options and Accessories listed in the Installation Section of this manual.

11.8. OPTIONS

11.8.1. Standard Configuration: The instrument is supplied with a column mounting panel, injector mounting bracket, and start-up kit in either a 50 or 60 Hz configuration.

Optional Configuration: The Series 4 is also available equipped with either or both a column oven and a high-speed injector valve.

11.9. INSTALLATION

The Series 4 Solvent Delivery System is field-sevice installed.

APPENDICES

Appendix A	LC Solvent Polarity and Property Scale
Appendix B	Ultraviolet Absorption Bands of Common Chromophores
Appendix C	Column and Solvent Selection Guide
Appendix D	Common LC Calculations
Appendix E	The General Elution Problem and Its Solution
Appendix F	Fundamental Concepts of Liquid Chromatography
Appendix G	Use of the Solvent Gradient

APPENDIX A
LC SOLVENT POLARITY AND PROPERTY SCALE

Solvent	Adsorptive Energy	Boiling Point, °C	Viscosity	Refractive Index	UV cutoff/nm
*Hexane	0.00	69	0.33	1.375	210**
*Isoctane	0.01	99	0.3	1.404	210**
Petroleum ether Skellysolve B, etc.	0.01	175-240	0.3		210
n-Decane	0.04	174	0.92	1.412	
Cyclohexane	0.04	81	1.00	1.427	210
Cyclopentane	0.05	49	0.47	1.406	210**
Carbon tetrachloride	0.18	77	0.97	1.466	265
Amyl chloride	0.26	108	0.43	1.413	225
*Butyl chloride	0.26	77	0.47	1.436	220
Xylene	0.26	138-144	0.62-0.81	~1.50	290
*i-Propyl ether	0.28	69	0.37	1.368	220
Toluene	0.29	111	0.59	1.496	285
Chlorobenzene	0.30	132	0.80	1.525	330
Benzene	0.32	80	0.65	1.501	280
*Chloroform	0.40	61	0.57	1.443	245
Methyl-i-butylketone	0.43	117		1.394	330
*Tetrahydrofurane	0.45	65	0.35	1.408	220
Ethylene dichloride	0.49	84	0.79	1.445	230
Methylethylketone	0.51	80	0.3	1.381	330
Acetone	0.56	57	0.32	1.359	330
*Dioxane	0.56	101	1.54	1.422	220
Ethyl acetate	0.58	77	0.45	1.370	260
*Amyl alcohol	0.61	138	4.1	1.410	210
Dimethyl sulfoxide	0.62	189	2.24		
Nitromethane	0.64	101	0.67	1.394	380
*Acetonitrile	0.65	82	0.37	1.344	210**
Pyridine	0.71	115	0.94	1.510	305
*i-propanol, n-propanol	0.82	83	2.3	1.38	210
Ethanol	0.88	79	1.20	1.361	210
*Methanol	0.95	65	0.60	1.329	210
Ethylene glycol	1.11	198	19.9	1.427	210
Acetic acid	Large	118	1.26	1.372	
*Water	Larger	100	1.0	1.333	190**
Salts & Buffer	Very Large	100+			

List of common chromatographic solvents in order of increasing polarity with RI values.
From "Principles of Adsorption Chromatography" L.R. Snyder, Dekker.

*Most commonly used with U.V. Detector.
**Extremely pure grades may be used as low as 195 nm.

The Hildebrand solvent scale is a list of common solvents used in liquid Chromatography in order of increasing energy of adsorption on alumina. The values are different, but the order is essentially the same on silica gel as on alumina.

The starting solvent selected for a given separation can be chosen by matching the relative polarity of the solvent to that of the sample. This is done as a first approximation by selecting the solvent to match the most polar functional group on the sample molecule. (eg alcohols of OH, amines for NH_2 etc.) From this attempt the separation can be refined by the following procedure.

1. If the sample appears at the solvent front then the solvent is too polar to allow the adsorbent to retard the sample. Go to a solvent higher up (lower polarity) on the scale.

2. Conversely if the sample does not appear in a reasonable time go to a solvent or solvent blend lower down (higher polarity) on the scale.

Solvent blends are most useful when they are composed of pairs which differ in adsorption energy by no more than 100%. (Example propanol at energy 0.82 and chloroform at energy 0.40)

Solvent gradients should be generated on the same basis of no more than doubling the energy of the starting solvent. The range is more than sufficient for most if not all L C separations.

When changing solvents or when regenerating after a gradient, a minimum of 5 column volumes of the new starting solvent should flow through the system before another sample injection is made.

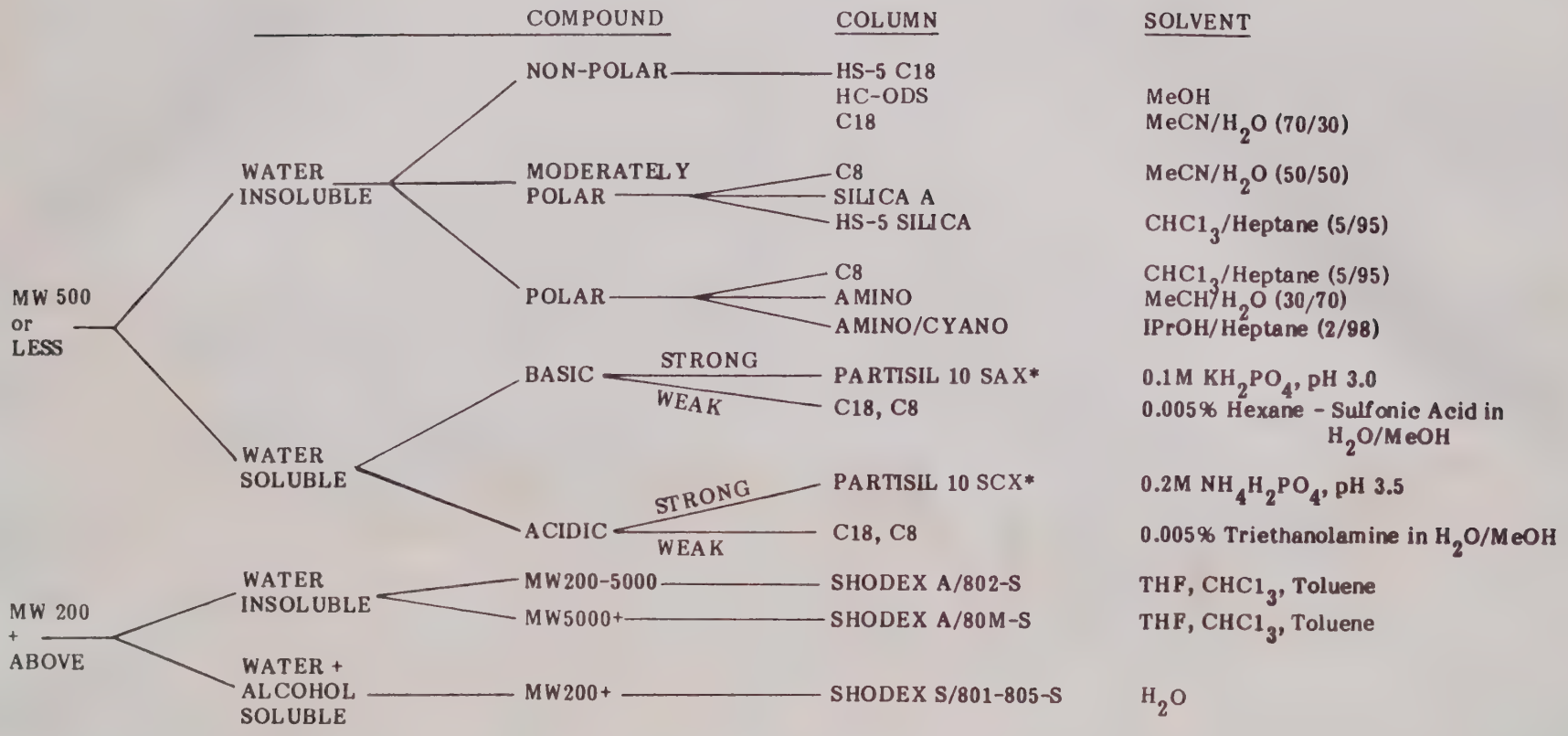
Notes on solvent miscibility
Normally, solvents are completely miscible if they are on the same half of the scale. In other words, all solvents on the upper half of the scale are completely miscible, or all on the bottom half are miscible with each other, and all solvents from the center half (1/4 to 3/4) are mutually miscible. There are several "universal" solvents such as tetrahydrofurane and acetonitrile which are miscible with almost all others except hexane and pentane. By far the most useful "rinsing, or cleanout" solvent is isopropanol, which is miscible with all others at all concentration levels.

APPENDIX B
ULTRAVIOLET ABSORPTION BANDS OF COMMON CHROMOPHORES

CHROMOPHORE	SYSTEM	λMAX	εMAX	λMAX	εMAX	λMAX	εMAX
Ether	-O-	185	1000				
Thioether	-S-	194	4600	215	1600		
Amine	$-NH_2$	195	2800				
Thiol	-SH	195	1400				
Disulfide	-S-S-	194	5500	255	400		
Bromide	-Br	208	300				
Iodide	-I	260	400				
Nitrile	-C≡N	160	-				
Acetylide	-C≡C-	175-180	6000				
Sulfone	$-SO_2-$	180	-				
Oxime	-NOH	190	5000				
Azido	>C=N-	190	5000				
Ethylene	-C=C-	190	8000				
Ketone	>C=O	195	1000	270-285	15-30		
Thioketone	>C=S	205	strong				
Esters	-COOR	205	50				
Aldehyde	-CHO	210	1500				
Carboxyl	-COOH	200-210	50-70				
Sulfoxide	>S→O	210	1500				
Nitro	$-NO_2$	210	strong				
Nitrite	-ONO	220-230	1000-2000	300-400	10		
Azo	-N=N-	285-400	3-25				
Nitroso	-N=O	302	100				
Nitrate	$-ONO_2$	270 (shoulder)	12				
	$-(C=C)_2-$ (acyclic)	210-230	21,000				
	$-(C=C)_3-$	260	35,000				
	$-(C=C)_4-$	300	52,000				
	$-(C=C)_5-$	330	118,000				
	$-(C=C)_2-$ (alicyclic)	230-260	3000-8000				
	C=C-C≡C	291	6,500				
	C=C-C=N	220	23,000				
	C=C-C=O	210-250	10,000-20,000			300-350	weak
	$C=C-NO_2$	229	9,500				
Benzene		184	46,700	202	6,900	255	170
Diphenyl				246	20,000		
Naphthalene		220	112,000	275	5,600	312	175
Anathracene		252	199,000	375	7,900		
Pyridine		174	80,000	195	6,000	251	1,700
Quinoline		227	37,000	270	3,600	314	2,750
Isoquinoline		218	80,000	266	4,000	317	3,500

APPENDIX C

COLUMN AND SOLVENT SELECTION GUIDE



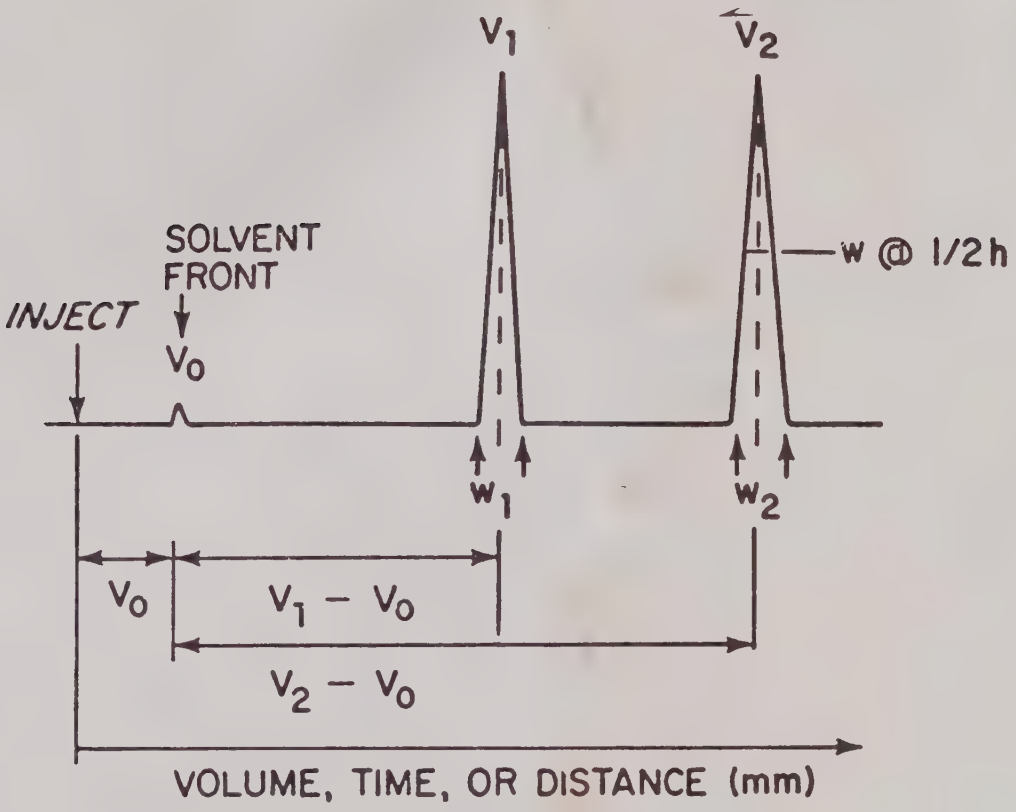
*Reverse Phase Ion Pairing may in some cases be substituted for Ion Exchange. See the Chart on the reverse side of this page or refer to: R.W. Yost, L.E. Ettre and R.D. Conlon, Practical Liquid Chromatography: An Introduction. Norwalk, Ct.: Perkin-Elmer 1980.

COLUMN/SOLVENT/COMPOUND GUIDE ***

Compound Ionization	Strongly Acidic	Slightly Acidic	Neutral	Slightly Basic	Strongly Basic
MODE	1. ion exchange or 2. ion pairing	1. ion suppression or 2. ion exchange or 3. ion pairing	1. Normal Phase or 2. Reverse Phase or 3. GPC	1. ion exchange or 2. ion pairing or 3. ion pairing	1. ion exchange or 2. ion pairing
MOBILE PHASE	1. *Ion Exchange Mobile phase; Buffer Example: .01M NaH_2PO_4 Mechanism example ion exchange particle $-NH_3^{(+)}$ $OH^{(-)}$ / $R-SO_3^{(-)}H^{(+)}$ To elute compounds, vary pH or ionic strength of buffer 2. **Ion Pairing Mobile phase: example: water/methanol modified with .005M tetrabutyl ammonium hydroxide (TBA) and proper buffer To elute compounds, vary methanol (containing TBA) concentration.	1. Ion Suppression add acid such as .01-.1 phosphoric acid or 3 acetic acid to mobile phase. Use appropriate precautions. 2. Ion Exchange See strongly acidic column. 3. Ion Pairing See strongly acidic column.	Unmodified Mobile Phase Examples: 1. Normal Phase Hexane/Chloroform 2. Reverse Phase Water/Acetonitrile 3. GPC Tetrahydrofuran	1. Ion Suppression add base such as .01-.1% R_2-NH or NH_4OH to mobile phase. Use appropriate precautions. 2. Ion Exchange See Strongly Basic Column. 3. Ion Pairing See Strongly Basic Column	1. *Ion Exchange Mobile phase; Buffer Example: 0.0 1m Na_2HPO_4 Mechanism example ion exchange particle $-SO_3^{(-)}\rightarrow H^{(+)}$ / $R-nH_3^{(+)}$ $x^{(-)}$ To elute compounds, vary pH or ionic strength of buffer 2. **Ion Pairing Mobile phase: Example: Water/Methanol modified with 0.005m hexane sulfonic acid (HSA) and proper buffer To elute compounds, vary methanol (containing HSA) concentration.
COLUMN	1. *Anion Exchange 2. **C18	1. C18 or Silica 2. Anion Exchange 3. C18	1. Silica 2. C18 3. Shodex	1. C18 or Silica 2. Cation Exchange 3. C18	1. *Cation Exchange 2. **C18

*** For specific column operation with solvents such as acids, bases and buffers, please refer to each manufacturer's specific instructions and warnings regarding operation with acids, bases and buffers.

APPENDIX D
COMMON LC CALCULATIONS



THEORETICAL PLATES(N) = $16\left(\frac{V}{w}\right)^2$ or $5.5\left(\frac{V}{w\ @\ \frac{1}{2}h}\right)^2$

HEIGHT EQUIVALENT TO THEORETICAL PLATE(HETP) = $\frac{\text{COL. LENGTH(mm)}}{N}$

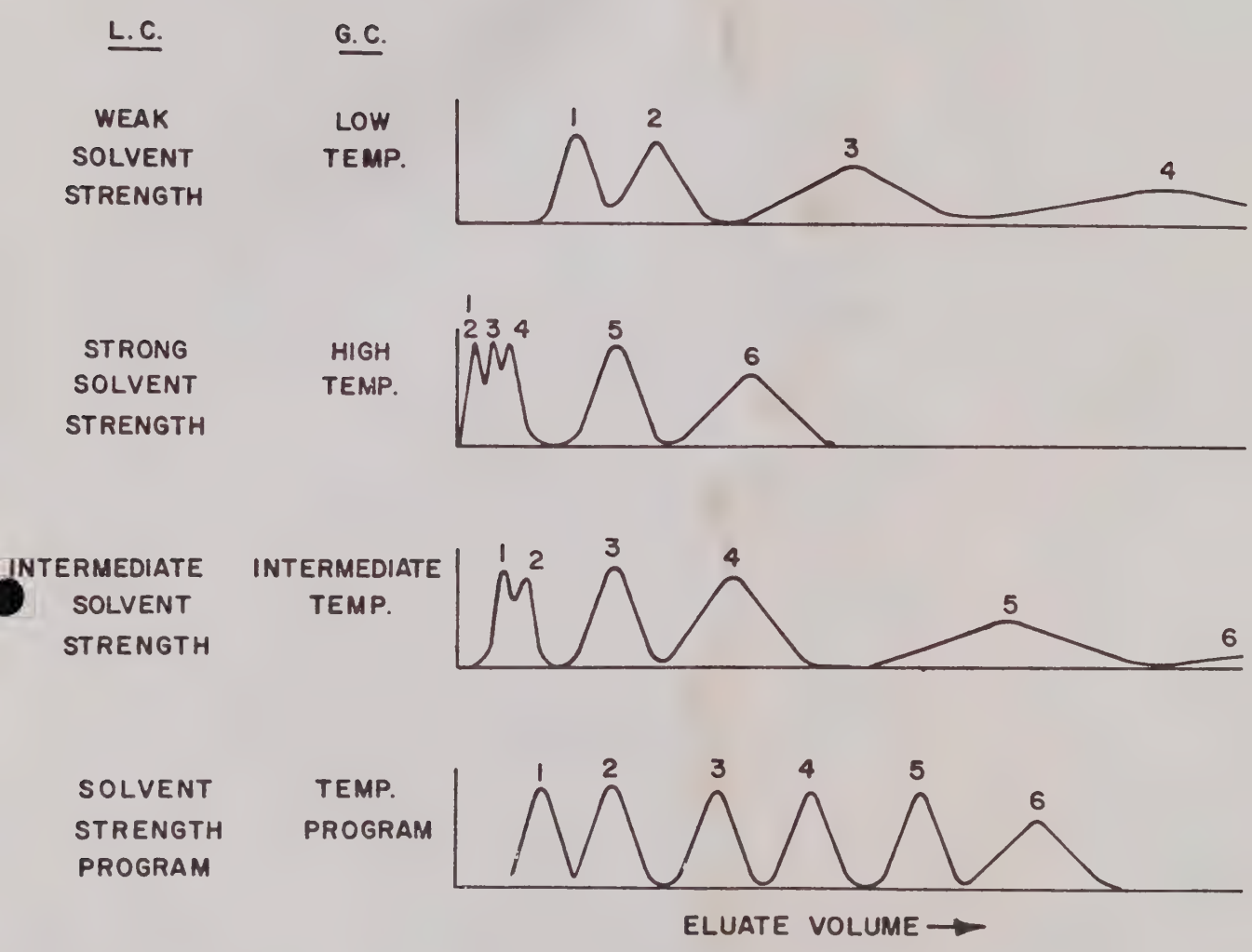
RESOLUTION (R_s) = $\frac{V_2 - V_1}{\frac{1}{2}(w_2 + w_1)}$

CAPACITY FACTOR (k') = $\frac{V_1 - V_o}{V_o}$ and $\frac{V_2 - V_o}{V_o}$

SEPARATION FACTOR(α) = $\frac{k'(V_2)}{k'(V_1)}$

APPENDIX E

THE GENERAL ELUTION PROBLEM AND ITS SOLUTION



APPENDIX F

FUNDAMENTAL CONCEPTS OF LIQUID CHROMATOGRAPHY

INTRODUCTION

Liquid chromatography, an automated, high resolution approach to column chromatography, provides the researcher with yet another separation mechanism to solve analytical problems. It enables the user to make fast, efficient separations, many previously unattainable, under mild conditions on thermally-labile and high molecular weight compounds. The potential usefulness of liquid chromatography far surpasses that of gas chromatography. While gas chromatography is applicable to approximately 15% of the known compounds, liquid chromatography is useful in analyzing the remaining 85%. In order to achieve this capability, a liquid chromatograph must be designed to allow the user to perform readily in all four modes of liquid chromatography: Normal Phase (Adsorption), Reverse Phase (bonded phase), gel permeation and ion exchange chromatography.

THE BASIC LIQUID CHROMATOGRAPH

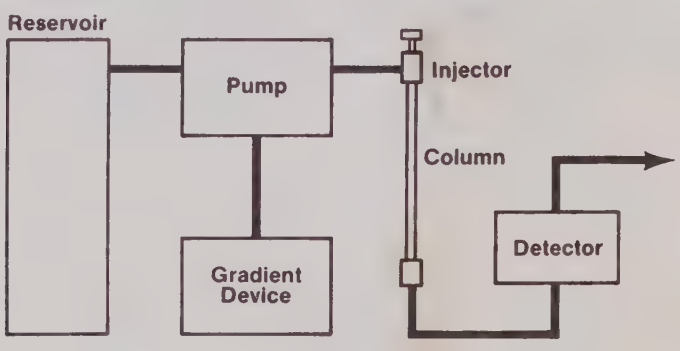


Figure F-1

The basic liquid chromatograph consists of four elements. These are: a pump to move the solvent carrier through the system, an injector for depositing the sample at the head of the column, the column filled with a packing material, and a detector to visualize the eluted components.

For separation of complex mixtures and to shorten analysis time, gradient elution, or solvent programming capability, is required. Solvent programming is the most powerful of all liquid chromatography parameters for increasing efficiency and reducing analysis time.

The design goals of a research liquid chromatograph must be dictated by the chemistry of separation and the physics of fluids in motion, to achieve resolution of the individual components of the sample and retain this resolution until the separated species are detected and possibly collected. The pump should provide a constant

pulse-free flow of solvent. The pressure increases as additional lengths of column are installed to increase resolution. Although different solvents have widely varying viscosities, when changing solvent systems, the researcher does not want flow rate changes due to viscosity changes. Constancy and repeatability of flow rate, however, are essential in order to employ elution volume (or elution time at constant flow rate) in component identification. Smooth constant flow of solvent carrier is also necessary for best detector performance since many photometric detectors exhibit drift and noise with long- and short-term changes in flow rate.

Injection devices and column end fittings must be designed with utmost care since void space in liquid chromatography has much more effect on peak broadening than in gas chromatography.

Columns require a smooth precision bore and uniform particle size packing. Finally, detectors must have highly efficient flow cells which allow the separated sample components to pass through in a minimum time. Other essential characteristics are low cell volume, high sensitivity and stability, and high pressure cells, which allow backpressure to be put on the cell to prevent bubbles of dissolved gas from developing in the cell.

LC column packings are extremely small and packed tightly into the column. They offer a high amount of resistance to the flow of liquids past and through them. This results in relatively significant amounts of backpressure at operating flow rates.

MODES OF LIQUID CHROMATOGRAPHY

LIQUID-SOLID (ADSORPTION) CHROMATOGRAPHY

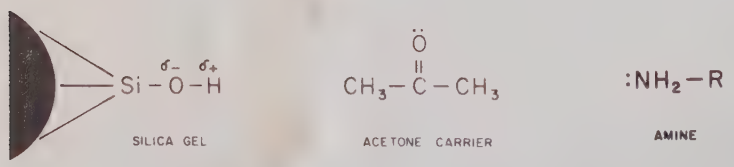


Figure F-2

The separation mechanism in liquid-solid (adsorption or normal phase chromatography) is based on the competition between the sample and the solvent molecules for the active sites on an adsorbent such as alumina or silica gel. Application is to molecules of low to high polarity up to 1000 molecular weight. Typical column lengths are 0.15 to 0.25 meters and 2 to 4.6 mm I.D. Backpressure at a flow rate of 1 ml/min. is about 1000 psi per 15 cm length with 5 μm diameter packing. Mixed solvent capability is essential and gradient elution capability is useful

to application of this mode since most separations are achieved by controlling the adsorptive energy of the eluent. This control is normally effected by blending, or modifying, a weak (non-polar or low adsorptive-energy) solvent with a stronger solvent, as described on the Solvent Polarity Scale (Appendix A). Since most adsorptive columns retain a coating of the more polar solvent, the separating mechanism may actually be the result of the sample partitioning between the eluting solvent and the modifying solvent which is being retained on the column. (See below.) Guidelines for solvent selection and solvent concentration adjustment are provided in Appendix A.

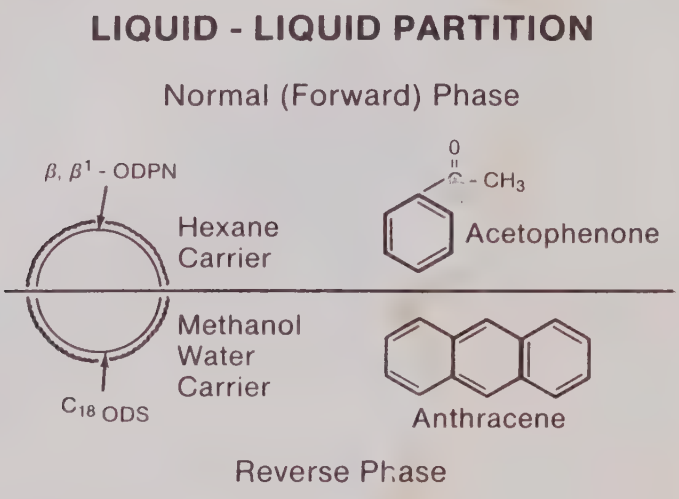


Figure F-3

There are two basic modes of partition chromatography: normal or forward phase, where the column surface is polar and the eluent is much less polar, as in adsorption chromatography; and reversed phase where the column surface is non-polar and the eluent is polar (usually water modified with an alcohol or less-polar organic, such as acetonitrile). Most modern HPLC is performed on permanently-bonded stationary phases, where the stationary phase is chemically bonded to the packing. The 18-carbon octadecylsilane (ODS) bonded column is one of the most widely-used types, since these columns, while ideal for non-polar samples, may also be used for moderately polar and polar samples. More recently, the shorter-chain, 8-carbon (C8) columns have been recommended for these more-polar samples. As with the C18 (ODS) columns, water is commonly used as the "weak" solvent and methanol or acetonitrile is added to speed elution of strongly-retained compounds. Some organic soluble compounds may be so strongly retained that the solvent system may use acetonitrile as the weak solvent with THF or methylene chloride added to speed elution.

Separation in liquid-liquid partition (or reverse phase) is based on the comparative solubility of the sample molecule in the solvent and the liquid coating on a solid support. Partitioning the sample between phases delays or retains some components more than others to effect separation. Application is to moderately polar substances

up to molecular weight 1000. Columns of 0.10 to 0.25 meter length (2 to 4.6 mm I.D.) are normally used. Typical backpressure is 1000 psi per 15 cm length at 1 ml/min. with 5 μm diameter packing. The technique requires a constant solvent flow rate independent of backpressure to permit identification on the basis of retention volume.

EXCLUSION CHROMATOGRAPHY

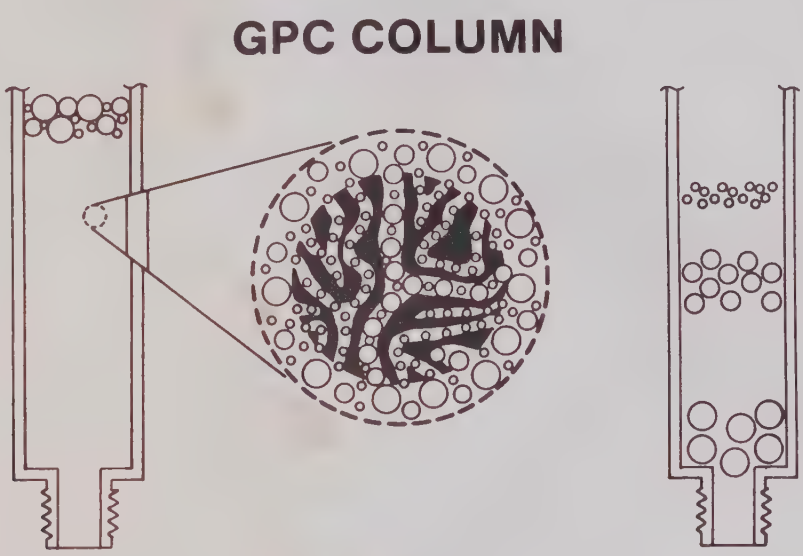


Figure F-4

The mechanism of separation in exclusion chromatogrpahy is based on the physical size of sample molecules. The column is packed with a gel having pores similar in size to the molecular dimensions of the sample. Small molecules are able to permeate more pores and are therefore retained longer than large molecules. Molecular weight range of application is 200 to several million. Column lengths of from 25 cm to a meter or more are used. Backpressures at a flow rate of 1 ml/min. are in the range of 150 psi. This technique is applicable to polymers and other higher molecular weight samples and requires a constant, highly reproducible solvent flow rate since time of emergence of species is inversely proportional to molecular size and systems are frequently calibrated by constructing a plot of retention time vs. log molecular weight. Recently, columns have become available which allow high resolution of relatively low molecular weight materials and these can be used to pre-separate samples into high- and low-weight fractions which are further separated by either normal or reverse phase techniques.

ION EXCHANGE

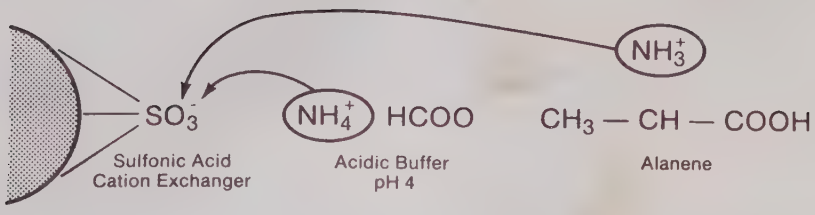


Figure F-5

Separation in ion exchange chromatography is based on the competition between the buffer solvent and the ionic or highly polar sample for sites on the ion exchange resin column packing. Application is made to a wide range of molecular weights. Peptides and amino acids are traditional examples. This technique requires 0.10 to 0.5 meters of 2 to 4.6 mm I.D. column. Typical backpressures are 800 to 3000 psi depending on the speed of the analysis. Ionic and/or pH gradients are essential to sweep the sample components from the column in a reasonable time.

INSTRUMENTAL REQUIREMENTS

PUMP DRIVE SYSTEM

Pumping systems can be extremely simple, where one pump is used to provide a single solvent or pre-mixed solvent blend, but more typically, can be quite complex, where the blending of several solvents can be achieved by the pumping system. Microprocessor-controlled pumping systems can produce solvent blends and solvent programs or gradients with an infinite variety of compositions and flow rates.

SINGLE SOLVENT

The chromatographic system employing a single solvent can be used in all chromatographic modes where adequate separation is achieved with an isocratic solvent system. Essential requirements are absolute constancy of flow rate and the ability to repeat previously-used flow rates in order to use the parameter of elution volume or time to identify species or to calculate molecular weight. Of course, as described above, a simple pump can be used to deliver an eluent which is a mixture of solvents (isocratic analysis) composed to obtain the required separation. If the concentration of the mixture is improper, however, it is fairly inconvenient to change in a pump which does not allow the user to blend solvents, requiring either dilution of the original, or mixing of a new blend.

MIXED SOLVENT

Mixed solvent operation is used primarily in normal phase and reverse phase chromatography. It is also useful in ion exchange work. The ability to alter the polarity of the carrier by changing the blend ratio of different solvents saves hours of mixing and solvent changeover time. The essential parameter is constancy of flow rate despite rapid changes in carrier viscosity and the resultant changes in column backpressure.

The Series 4 pump can be charged with up to four different solvents or different ionic strength buffers and the precise fraction of blended solvent desired can be programmed. After a preliminary run, corrections can be made on the choice of solvent blend to improve resolution and to decrease analysis time. The precent of A in A+B+C+D can be varied during a run to save time in sweeping strongly retained species from the column. This gives preliminary information on the type of gradient which may be helpful in the analysis. Additionally, any ratio can be selected at any flow rate.

Mixed solvent systems are of great use to the chromatographer. When a non-polar solvent with adsorbent packing is used, the solvent does not readily displace polar sample molecules adsorbed on the packing, and retention times can be extremely long. With the use of a polar solvent, however, the sample molecule is readily displaced from the packing, and retention time decreases. When a mixture of two or more solvents is blended, the retention times can be increased until all sample components are separated in a minimum of time.

SOLVENT PROGRAMMING

The solvent gradient is an essential capability in ion exchange, normal phase and reverse phase chromatography when samples cover a broad polarity range as noted in Appendix E. The solvent or buffer carrier must be changed in a predictable and highly reproducible manner in order to sweep the sample components from the column with maximum resolution and minimum time. This requires precise control of the pump system to achieve flexible, reproducible gradient profiles and a constant solvent flow rate despite changing solvent viscosity and the resultant change in column backpressure. Additionally, there must be no dead volume where unmixed solvent can be retained.

While more simple units allow the use of strictly linear program changes, where the rate of change, or total time, is the only variable, more complex instruments such as Series 4 include curved gradient capability.

Solvent gradients, from convex through linear to concave, can be selected and adjusted through a wide range of curvatures. Convex, linear, and concave gradients and the degree of curvature are selected at the LC Terminal. Since the gradients are generated by digital techniques, the long term precision of the system is assured.

To form a solvent gradient, the reservoirs are filled with solvents of different polarity or ionic strength which represent the limits which should be necessary to remove all the sample species from the column. The LC Terminal is used to select the solvent percentage for each of 5 separate time segments so that the flow rate is

held constant while the solvent percentages change. The output solvent composition is smoothly and reproducibly varied by any linear or non-linear function. In some applications, the solvent concentration is held constant and the flow rate is programmed, beginning with a very slow flow to elute weakly-retained species, and gradually increasing to elute more strongly-retained components. The Series 4 permits changing both flow rate and composition at the same time if required.

COLUMNS AND INJECTORS

Radial diffusion in gas chromatography systems tends to reduce transcolumn effects. That is, when moving from one diameter conduit to another, radial diffusion helps sweep the material out of the corners. Radial diffusion in a liquid stream is only about 1/10,000 of that in a gaseous stream, however. For this reason, conduits must be kept as small and as constant as possible, and in zones where the conduit diameter must change, special consideration must be given to the zone of transition from one diameter to the other.

The method of injection is naturally critical in liquid chromatography. Several methods are in common use. The first is a high pressure loop injector available with variable volume loops and fixed internal loop versions available in the range of less than a microliter to several milliliters. These injectors are designed with a uniform bore throughout the injector body, seat, and loop to eliminate back mixing of the sample. Other valve injectors allow the applications of various amounts of sample without loop change. These injector values are loaded by means of a hypodermic syringe.

DETECTORS

The design of liquid chromatography detectors requires more than just high sensitivity. Low dead volume is necessary to prevent unwanted diffusion or band spreading. The basic cell design must be of an efficient nature to allow rapid flushing with minimal volumes of solvent. Dynamic range must be wide to provide the high sensitivity required in analytical work while retaining the ability to be attenuated on-scale with large preparative samples. Finally, a high degree of stability is required since this determines the accuracy of quantitation.

UV DETECTORS

The most common LC detectors are those which detect changes in ultraviolet (UV) absorption. Typically these include an energy source beaming light through a long, narrow flowcell through which column effluent is pumped. Non-absorbing solvents are used so the normal baseline represents maximum energy resulting from all of the light reaching the photodetector which is on the opposite side of the cell from the light source. Some light is lost when compounds which have various types of molecular structure, or contain functional groups which absorb UV light at the wavelength in use, pass through the cell. The resultant change in energy is converted to an increase in millivolt output which is then displayed on a strip chart recorder and/or is integrated on data acquisition devices.

The greater the concentration of the absorbing compound in the cell, the more light is absorbed, thus the larger the signal. In addition, since the degree of absorption is dependent upon the specific structure or functional groups present (molar absorptivity), signal intensity is also a function of the type of compound being detected. This can vary by a factor of thousands, as noted in Appendix B. Thus, strict standardization procedures must be used to determine the actual response of each component for quantitative work.

UV detectors range from simple single wavelength units which obtain energy primarily at 254 nm (where about 60% of all organics have some absorption) from low pressure mercury lamps; to more widely applicable monochromator-equipped units utilizing broad-spectrum deuterium light sources. With these latter units any wavelength between 190 and 650 nm may be selected to take advantage of the optimum UV absorption characteristics of specific sample species. (See Appendix B.) This allows detection of over 90% of all organic compounds.

The Perkin-Elmer UV detectors were designed with low volume, high efficiency cells coupled to highly stable electronics possessing wide dynamic range to provide the ultimate in liquid stream detection. Additional benefits include high pressure capability which allows backpressure to be placed on the cells, often eliminating the need for solvent degassing.

REFRACTIVE INDEX DETECTORS

Another detection mode is Refractive Index, where a dual-cell detector allows continuous comparison of a reference stream, containing only eluting solvent, with the sample stream. As sample components elute, they refract light at different angles, resulting in changes of energy at the photodetector, and in changes in output signal as described previously. While the UV detector ideally measures changes of light energy in a single cell, and requires no equilibration or flushing, the RI detector is strictly a comparative technique, where the output is always the difference between the two cells. It is therefore difficult, or pratically impossible, to change solvents during runs, and requires that the reference side be purged and re-equilibrated each time the slightest solvent concentration change is performed. The use of solvent gradients is therefore prohibited with RI detectors.

FLUORESCENCE DETECTORS

About 5 to 10% of the known organic compounds fluoresce (emit high-wavelength energy when excited by low-wavelength energy). When this property exists, fluorescence detectors, which allow selection of optimum excitation and emission wavelengths (as described in Perkin-Elmer's publication "Introduction to Fluorescence" [TN-60], provide high degrees of selective and sensitive detection.

DETECTOR COUPLING

If zero dead volume unions and extremely narrow-bore tubing are used, (0.015-inch or less) detectors may be coupled in series. Ideally, since its cell can withstand more backpressure than RI or fluorescence detectors, the UV detector should be the first, RI the second, and fluorescence the third detector in the coupled series.

APPLICATION

While the RI detector is most general (everything has a refractive index), maximum sensitivity is limited to about one microgram of material. Fluorescence detection is most sensitive, with strong-fluorescing compounds detectable to the sub-picogram level, but is the most limited in its application. The best combination of applicability and sensitivity is found in the variable-wavelength UV detector where typical detection capability is in the nanogram region for most strong-absorbing compounds.

TEMPERATURE CONTROL

Increasing the effective operating temperature in LC has two important effects. First, mass transfer between the phases is increased, resulting in sharper and more symmetrical peaks (better separations). Second, the unique retention time for each sample component will be decreased; i.e., it will elute from the column more quickly. However, a given temperature change will not affect each sample component to the same degree.

Temperature control is essential for achieving reliable and reproducible separations by liquid chromatography. It is an important variable that can be used routinely to:

- provide better quality separations through improving both column efficiency and peak symmetry;
- optimize separation time. Elevated temperatures reduce solvent viscosity and therefore generally permit the use of higher flow rates (shorter run times) within backpressure limitations;
- increase the reliability of qualitative analysis in LC based on peak retention times by making the separation independent of varying ambient temperature.

Common operating temperatures in LC are in the 60 to 79 degree centigrade region. This is normally sufficient to achieve the desired enhancement of solubility, reduction of viscosity, and elimination of ambient-change-induced variances.

SAMPLE PREPARATION AND QUANTITATION

Many samples may be simply dissolved in a suitable solvent (often the mobile phase) and injected directly into the instrument. If samples contain particulate material, or components which may bond to or alter the column (such as the proteins in plasma and urine samples) they should be either filtered through a micro-porous filter such as the Perkin-Elmer Filter No. 0089-0835 or Filter No. 0089-0837 (see PL-409) and/or extracted or filtered through ion-exchange media. Sample and solvent workup procedures are thoroughly discussed in Perkin-Elmer's publications TN-69 and LC-100. LC-100 also includes information on the use of LC in quantitative analysis.

PREPARATIVE SCALE OPERATION

After successful analytical separation of a sample is achieved, it is often required that the analysis be scaled up to allow sufficient material to be processed so that subsequent analysis for identification or experimentation can be performed. This mode of operation requires large column capacity, increased solvent capacity, and sample collection capability. Solvent flow rate should be constant, and sample collection actuation must operate on a precise time base. This allows immediate and non-ambiguous identification and location of the sample components in the corresponding vials of the fraction collector, referenced to the recorder trace of the detector output.

SELECTION OF SOLVENT AND DETECTION WAVELENGTH

UV absorption spectra of compounds can be used to determine optimum wavelength settings. Compound A may be seen to have high absorption at 260 nm; Compound B, at 330. Both have good absorbance at 255; thus this may be the best "compromise wavelength" if both are to be detected in the same run. Note that both have very high absorption in the far UV. If the solvent system contains chloroform, however, it can be seen that the chloroform would saturate the system below 230; thus, the far UV cannot be used in this case. If maximum detection of each compound is desired, it is sometimes useful to change wavelengths during the run. Note, too, that at 255 nm chloroform still absorbs some UV light while hexane does not. This means that a baseline shift would be noted if a solvent ratio change were performed.

APPENDIX G

USE OF THE SOLVENT GRADIENT

The solvent gradient, in liquid chromatography, is the most important single parameter for increasing resolution and reducing analysis time.

For optimum resolution and efficiency it is necessary to start with a solvent which is just good enough to dissolve the most soluble species in the mixture, and to end with a solvent just good enough to remove the most insoluble species from the column. Let us assume that these solvent limits are available. With complex separations the linear (Curve 1) gradient (as shown in Figure G-1) is attempted first, and refined subsequently by going to a concave or convex gradient if the separation demands.

Assume that an "early" stacking of unresolved peaks is shown in the chromatogram (see Figure G-2). This indicates that the rate of change of solvent composition early in the run was too rapid. A concave gradient (see Figure G-3) would improve the resoluiton. This is accomplished on the series 4 by entering a curve of 2, 3, 4, or 5. The curve will be chosen initially by determining the extent of stacking. The greater the stacking the greater the curve.

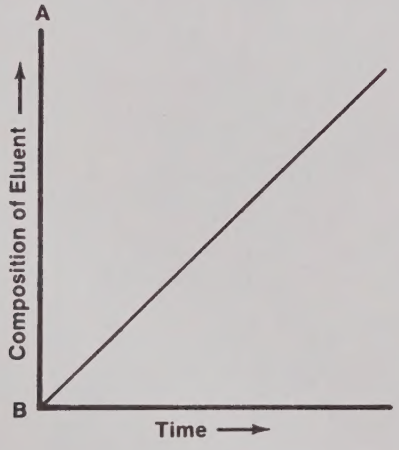


Figure G-1 - Linear Gradient (Composition of Eluent vs. Time)

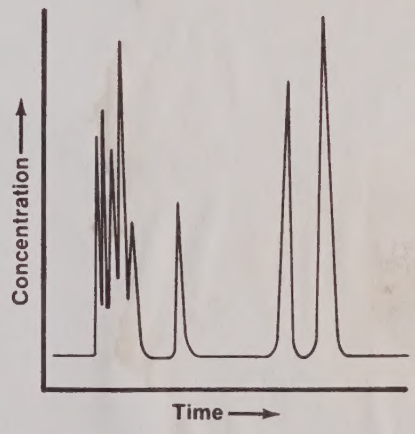


Figure G-2 - Early stacking of Unresolved Peaks

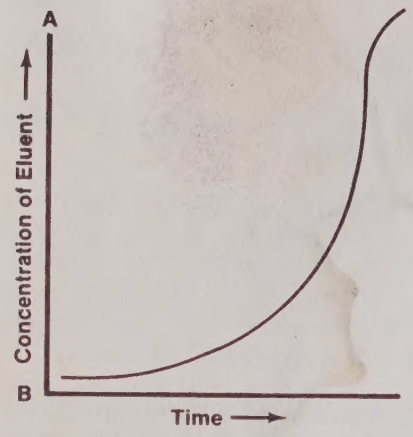


Figure G-3 - Concave Gradient

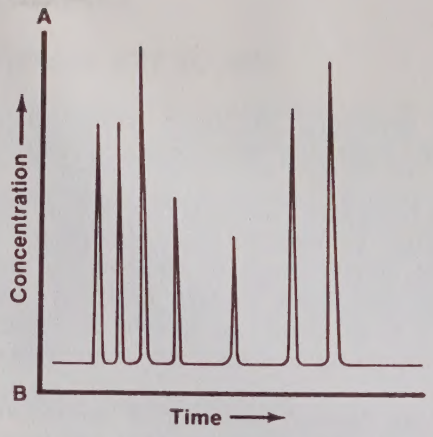


Figure G-4 - Ideal Resolution

If the stacking of unresolved peaks occurs late in the run (see Figure G-5), the initial part of the range of solvent compositions is not being utilized, and the rate of change in composition late in the run is too great. A convex gradient (see Figure G-6) allows a rapid initial change in composition to take place to reach the initial elution point of the mixture, followed by a low rate of solvent programming during the actual elution, thereby allowing better control of the separation. To obtain a convex gradient a curve of 0.2, 0.3, 0.4 or 0.5 should be used. The first peaks will then appear earlier and the last peaks later than those in Figure G-5, and a separation similar to the one shown in Figure G-4 will result.

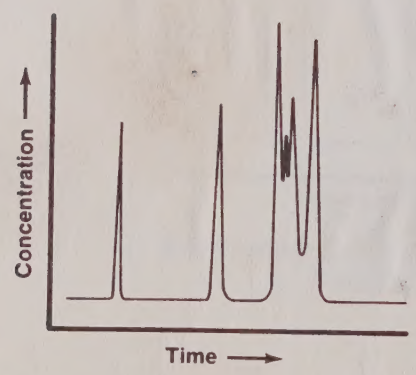


Figure G-5 - Late Stacking of Unresolved Peaks

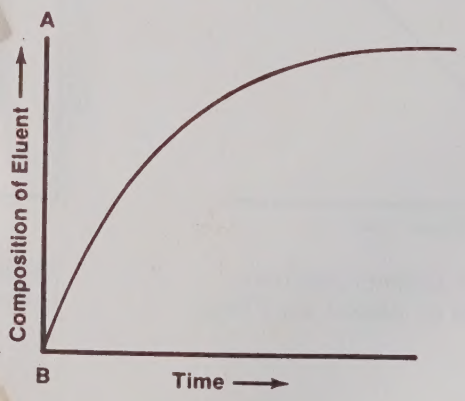


Figure G-6 - Convex Gradient

MP MASTER
MA32945